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PCR Amplification and DNA Sequence Analysis of the 45S Ribosomal DNA Cassette of *Chrysaora quinquecirrha*

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Abstract

The recent overpopulation of jellyfish occurring in the Barnegat Bay of New Jersey has been of growing concern. One prolific species, *Chrysaora quinquecirrha*, has been the subject of genetic research to determine the relatedness amongst populations and therefore determine its spread. In this study, a partial nucleotide sequence of the 45S ribosomal DNA cassette from *C. quinquecirrha* genomic DNA of Barnegat Bay was identified and compared to other cnidarian species. Approximately 44.8% of the cassette was identified, 3465 bp total. A partial 18S rDNA sequence was generated of 1772 bp (96% total). Complete ITS1, 5.8S rDNA, and ITS2 sequences were generated of 284 bp, 158 bp, and 205 bp, respectively. A partial 28S rDNA sequence was identified of 1046 bp long (29% total). It was found that the sequence of the 18S and 28S rDNA in *C. quinquecirrha* is closest to other *Chrysaora* species. The ITS regions of *C. quinquecirrha* of Barnegat Bay proved to be 99% identical to *C. quinquecirrha* of Navesink River, providing supporting evidence of how related these two populations of species are.

A complete cassette can potentially add another element when classifying and identifying organisms. Further studies to complete the 45S rDNA cassette will design specific primer sets to amplify the entire IGS region, the first 42 nucleotides at the 5' end of 18S rDNA, and the remaining 2560 bp of 28S rDNA. This data can then be utilized to determine the relatedness of other cnidarian populations and determine their spread.

MONTCLAIR STATE UNIVERSITY

/ PCR AMPLIFICATION AND DNA SEQUENCE ANALYSIS OF THE 45S
RIBOSOMAL DNA CASSETTE OF *CHRYSAORA QUINQUECIRRHA* /

By

Caitlin Michelle Burns

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A THESIS

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Chapter One: A Review of *Chrysaora quinquecirrha*

Taxonomy (Desor 1848)

Domain:	Eukarya
Kingdom:	Animalia
Phylum:	Cnidaria
Subphylum:	Medusozoa
Class:	Scyphozoa
Subclass:	Discomedusae
Order:	Semaeostomeae
Family:	Pelagiidae
Genus:	<i>Chrysaora</i> (Péron and Lesueur 1809)
Species:	<i>Chrysaora quinquecirrha</i> - sea nettle (Kramp 1961)

Genomics

Currently, little is known about the *Chrysaora quinquecirrha* genome. According to the NIH genetic sequence database (GenBank), the complete mitochondrial DNA sequence has been identified and sequenced (NC_020459.1; HQ694730.1), including the 16S rRNA gene (GU300724.2) (Park et al. 2012; Gaynor and Tare 2010). The beta-actin gene (JX409654.1), homeobox *scox6* gene (AY096262.1), homeobox *scox3* gene (AY096260.1), homeobox *scox1a* gene (AY096257.1), homeobox *neocox1Cc* gene (AY096244.1), Pax-B gene (U96197.1), Pax-A1 gene (U96195.1), and Pax-A2 gene (U96196.1) have also been identified. A partial 18S rRNA gene has also been identified (HM015266.1).

Mitochondrial DNA

Chrysaora quinquecirrha's mitochondrial DNA (mtDNA) is linear and is 16,775 bp long. Within this sequence, there are 13 protein coding genes necessary for oxidative phosphorylation, 16S rRNA and 12S rRNA, and three tRNA's: tRNA-Leu, tRNA-Ser(TGA), tRNA-Met (Hwang et al. 2013a). mtDNA has been used to indicate evolutionary and phylogenetic relationships among different species and even populations of species (Galtier et al. 2009).

Nuclear ribosomal DNA

Ribosomal DNA (rDNA) is the region of a genome coding for the RNA component of ribosomes. In eukaryotes, this DNA is generally found in tandem repeats, and can have over 10,000 copy numbers (Schlotterer 1998). Each copy of the 45S rDNA cassette is composed of genes coding for the 18S, 5.8S, and 28S rDNA, which are necessary for ribosomal functioning. Between these genes are two non-coding internal transcribed spacer (ITS) regions: ITS1 and ITS2. An intergenic spacer (IGS) connects each copy of the cassette together (Hillis and Dixon 1991).

The moon jelly, *Aurelia* sp. (EU276014.1), a scyphozoan, is currently the only cnidarian completely sequenced for the 45S rDNA cassette (Ki et al. 2009). According to Ki et al., the 45S rDNA cassette is 7731 bp long. The 18S rDNA gene is 1814 bp, ITS1 is 272 bp, 5.8S rDNA gene 158 bp, ITS2 is 278 bp, and 28S rDNA gene is 3606 bp long.

Only a partial 18S rRNA gene sequence has been identified for *C. quinquecirrha* (Gaynor et al. 2010). Many partial sequences of the 18S and 28S rDNA of other *Chrysaora* species have been identified, shown in Table 1.

Species	Accession	Location	Length
<i>C. fuscescens</i>	HM194815.1 HM194868.1	18S partial 28S partial	1764 bp 1066 bp
<i>C. melanaster</i>	HM194811.1 HM194864.1	18S partial 28S partial	1762 bp 1017 bp
<i>C. lactea</i>	HM1948.10.1 HM194863.1	18S partial 28S partial	1762 bp 1016 bp
<i>C. quinquecirrha</i>	HM015266.1	18S partial	520 bp
<i>Chrysaora</i> sp.	AY920769.1	18S partial	1797 bp
<i>Chrysaora</i> sp.	DQ083525.1	18S partial, ITS1 partial	276 bp
<i>C. colorata</i>	AF358098.1	18S partial	1830 bp

Table 1: Partial 18S and 28S rDNA identified sequences in *Chrysaora*. This table lists all current molecular sequence data available on GenBank for *Chrysaora* species.

Cnidarian Phylogeny

Many phylogenetic trees, which are hypotheses of evolutionary relationships, have been developed for cnidarians using various characteristics based mainly on morphology and more recently, genomics. There are four classes that make up the Medusozoa within the Cnidarian phylum: Hydrozoa, Cubozoa, Scyphozoa (of which *C. quinquecirrha* belongs to), and Anthozoa. Stalked jellyfish previously thought to belong in class Scyphozoa have recently been reclassified into a fifth class, Staurozoa (Marques and Collins 2004). Class Cubozoa, and Scyphozoa all exhibit an alternation of generations life cycle (a polyp and medusa stage), whereas Anthozoans only experience a polyp stage. Some species of from class Hydrozoa experience an alternation of generations and some do not. Two major hypotheses exist for the evolution of cnidarian life cycles (Fig. 1). One hypothesis is that the polyp-medusa life cycle originated from the polyp-only life cycle (Anthozoans). The second hypothesis is that the polyp-only life

cycle originated from the polyp-medusa life cycle, where classes Anthozoa, Scyphozoa, and Cubozoa are most closely related with one another and form a monophyletic group, and class Hydrozoa forms a paraphyletic group (Bridge et al. 1995).

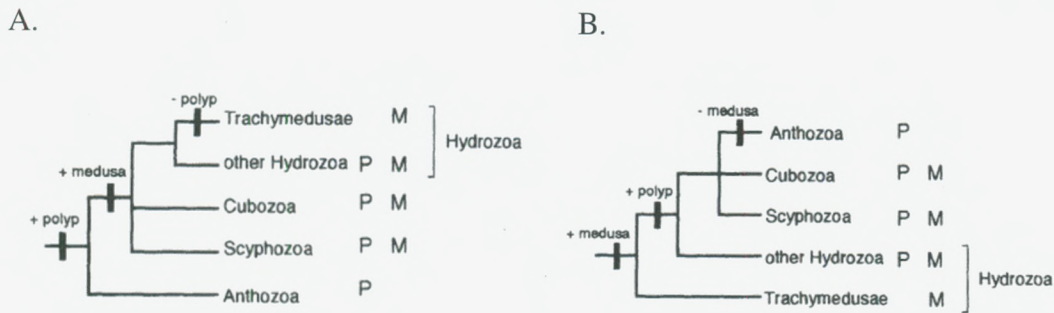


Figure 1: Evolutionary trees of two possible cnidarian life-cycle hypotheses. This image was taken from Bridge et al. (2005). Hypothesis 1 shown in A; Hypothesis 2 shown in B. P denotes polyp life form; M denotes medusa life form.

Morphological characteristics and mtDNA shape (circular or linear) was compared between 14 cnidarian species, 4 of which were Scyphozoans. Class Scyphozoa, Hydrozoa, and Cubozoa all have linear mtDNA, whereas class Anthozoa contains circular mtDNA. Results correlated with the first life cycle hypothesis where Scyphozoa, Hydrozoa, and Cubozoa are most closely related to one another, with Anthozoa as an outgroup (Schuchert 1993; Bridge et al. 1992).

Family Pelagiidae, of which *C. quinquecirrha* belongs to, was reclassified in 2002 by Gershwin and Collins. In this study, 20 morphological characteristics were analyzed between 15 pelagiid species (Fig. 2). This analysis hypothesized that *C. quinquecirrha* is most closely related to *C. pacifica*. In addition, all *Chrysaora* species are most closely related to one another when compared to other species in the family (*Sanderia* and *Pelagia*) with the exception of *C. achlyos*, which is most closely related to *P. colorata* (Gershwin and Collins 2002). More recently, rDNA has been investigated when

hypothesizing phylogenetic relationships among cnidarian species. However, with limited data available, it has been difficult to draw clear conclusions.

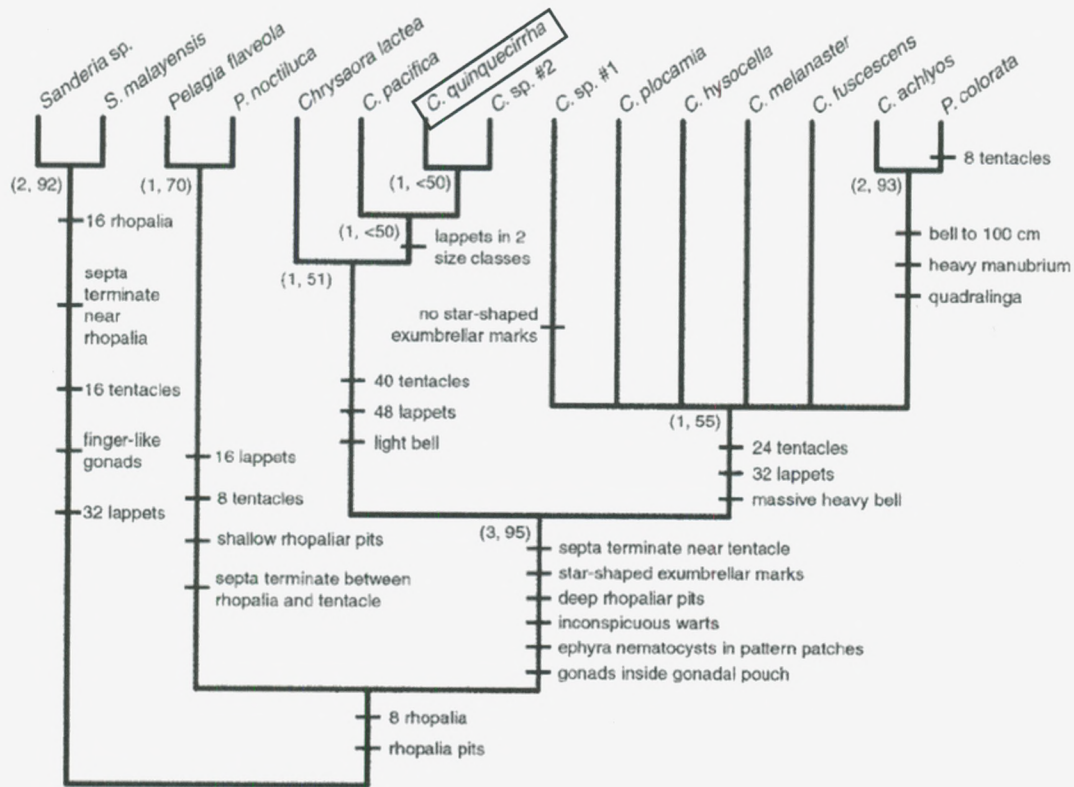


Figure 2: Phylogeny tree of family Pelagiidae species. This image was taken from Gershwin and Collins (2002).

Geographic Distribution

C. quinquecirrha is an aquatic species and is widely dispersed in tropical and temperate waters along the coasts of the Atlantic, Indian and Western Pacific Oceans. Along the East Coast of the United States, they can be found from southern New England to as far south as the Gulf of Mexico (Mayer 1910). They are reportedly abundant in the Chesapeake Bay, the largest estuary in the United States (Calder 1972a). More recently, they have populated the Barnegat Bay and Navesink River of New Jersey.

Sea nettles generally prefer waters of about 5-20 ppt salinity, whereas most other jellyfish prefer ocean water salinity of 35 ppt (Cargo and Schultz 1966). The optimal salinity for *C. quinquecirrha* is around 12 ppt (Purcell 2012). In the Chesapeake Bay, polyps are generally found in mesohaline waters, where salinity ranges between 5-18 ppt. Ephyrae are most commonly found in mesohaline waters as well. But, adult medusae can be found in both mesohaline and polyhaline (18-30 ppt) waters (Calder 1972b).

Anatomy

Chrysaora quinquecirrha, is classified based upon shared characteristics with other organisms. Sea nettles exhibit alternation of generations, where two main body forms exist: a small sessile polyp, which can be found throughout the year, and a larger free-floating medusa, or jellyfish, seen only during the warmer months of the year (Calder 1972b).

Chrysaora quinquecirrha have a simple tissue organization. They are diploblastic, where they originate from two primary germ layers: ectoderm and endoderm. The primary germ layers develop into two main epithelial layers: the gastrodermis, the innermost layer, and the epidermis, the outermost layer. The cells within each layer are connected to one another and have basement membranes. Between the epidermis and gastrodermis is mesoglea, a clear, jelly-like substance composed mostly of water and protein fibers, such as collagen, that acts to support the organism. This is where the term “jelly-fish” originates, due to its jelly-like mesoglea (Calder 1972a; Palomares and Pauly 2009).

Sea nettles are simple organisms and therefore do not contain brains, eyes, ears, hearts, or gills. Structures generally develop in multiples of four. They contain tentacles, sensory organs, a muscular system, and a simple nervous system. Body movements are coordinated by a nerve net and its associated sensory structures are found radially around the body. This allows the organism to detect and respond to a stimulus from any direction (Calder 1972a).

Both life history stages, polyp and medusa, contain one opening, where food is ingested and waste is released. The gastrovascular cavity is very simple and acts as both a stomach and intestines (Calder 1972b). It is divided into four sections, or septa. Water in the gastrovascular cavity is flushed by periodic body contractions and by currents from ciliated gastrodermal cells (Blanquet and Wetzel 1975). They also have radial symmetry, and their tentacles encircle the mouth/anus. Medusae contain four oral arms that are used to carry food into the mouth that has been trapped by its tentacles. Nematocysts are found on tentacles, which are stinging cells used to catch their prey (Calder 1972b).

During the polyp stage, *C. quinquecirrha* is very small, only about 0.5 to 3.5 mm in height and 0.6 mm in diameter. A polyp consists of three major components: a base, stalk and oral end. At the basal end of the organism is the base, which contains an adhesive pedal disc that attaches itself to a substrate. The stalk is the length of the organism and is cylindrical in shape. The gastrovascular cavity is found within the stalk. The oral end is located at the apical end of the organism. The oral end contains the mouth, which also acts as an anus for the digestive system. Tentacles of up to 6 mm in length contain nematocysts surround the mouth at the oral end (Littleford 1939).

During the medusa stage, *C. quinquecirrha* is inverted when compared to a polyp and exhibits an umbrella or dome-shaped body. It is composed of the two epithelial layers, mesoglea and tentacles. They can grow up to 25 cm in width and contain 8 tongue-shaped, semicircular lappets, or lobes, where the tentacles extend. The tentacles found along the outside rim of the umbrella are long, skinny and can grow up to 50 cm in length. The number of tentacles ranges: there are generally three large primary tentacles and two to four smaller ones (secondary and tertiary) within each octant. The lappet-clefts, or gaps, are deep in primary and secondary tentacles, but are shallow notches in tertiary tentacles. Each tentacle contains nematocysts. Four long oral arms are found extending from the middle of the umbrella and are used to carry food into the mouth. Their stomach pouches are of equal width. Septa, which are thin membranes in the stomach, are straight until they eventually make an S-like bend towards the rhopalar (sensory organ) radius, or margin. Along the rim of the umbrella is a ring of muscle fibers (within the mesoglea) that allow the organism to swim by contracting and relaxing these muscles. This allows the organism to propel itself through the water to catch its prey or avoid any predators. The color of *C. quinquecirrha*'s body can vary. They have been found milky white, yellow, and pink. Some can be found with red stripes traveling through the umbrella and tentacles (Calder 1972a, 1972b; Kramp 1961).

Cilia

In *C. quinquecirrha*, tiny hairs, or cilia are found throughout all areas of the epidermis at varying degrees (Blanquet and Wetzel 1975). Surrounding cilia at the base are microvilli. In a planula, cilia are found throughout the external surface of the

organism and contribute to their mobility. The cilia beat together and allow a planula to pulse through the water (Littleford 1939). The most heavily ciliated region of a polyp is the scyphopharynx-filament complex, which is the epithelium that lines the oral disc and surface of the gastric septae. Cilia are also heavily found on the tentacles and upper calyx. There is a strong correlation between where cilia are most often found and where a polyp is most likely to receive external stimuli. It is suggested that some cilia act as sensory receptors. All cilia are motile and produce a strong current that flows from basal to apical end of a polyp, carrying small particles away from the polyp. This protects the polyp from any accumulating material settling on and around it. A current also exists in the gastrovascular cavity, where the current travels towards the mouth to carry particles out of the polyp. This current aids in digestion because it allows ingested food to mix with digestive enzymes. The current also will remove any debris and undigested food from the gastrovascular cavity out of the mouth (Blanquet and Wetzel 1975).

Nematocysts

Cnidocytes are cells found on tentacles and around the mouth of a sea nettle and are used to capture prey and defend against predators. Many cnidocytes are grouped together in one location as a “battery” along with other supporting cells and neurons. Each cnidocyte contains a specialized organelle called a cnidocyst, or nematocyst. Nematocysts are secreted by the Golgi apparatus of a nematoblast, and are broken down into three components: a bulb-shaped capsule with a double-layered wall, a matrix with a hollow coiled stinging tubule, and an operculum, which resembles a trap-like door. On

the outside of a nematocyst is a cnidocil, a hair-like projection that acts as a trigger (Slautterback and Fawcett 1959; Holstein 1981).

Nematocysts are characterized by the presence or absence of spines on the tubules, their spine patterns, and tubule shape (Ostman 2000). Nematocysts differentiate into different types during strobilation (Calder 1977). There are two types of nematocysts found in *C. quinquecirrha*: atrichous isorhizas and heterotrichous euryteles (Calder 1972b). An atrichous isorhiza nematocyst tubule does not contain spines and is isodiametric, or spherical and uniform in shape. Heterotrichous eurytele nematocyst tubules have more than one type of spines and the tubule shaft is wider at one end and more narrow and constricted at the other end (Ostman 2000).

When a nematocyte is stimulated by mechanical (touch) and chemosensory stimuli, the thread shoots out through the operculum within three milliseconds via exocytosis and penetrates the prey and injects poison (Holstein and Tardent 1984; Oppegard et al. 2009). Prior to discharge, the venom is tightly coiled within the capsule. The coiled thread rotates as it discharges. High pressure within the capsule contributes to nematocyst discharge (Engel et al. 2002). Due to this high pressure, the capsule's wall is lined with minicollagens to provide strength for the nematocyst (Kurz et al. 1991; Holstein et al. 1994).

Life Cycle

Chrysaora quinquecirrha have a very complex life cycle, where they alternate between two main life forms (Fig. 3). They also have the ability to reproduce both sexually and asexually, which allows for their very high population numbers and survival

rate. During the medusa stage, organisms reproduce sexually, but during the polyp stage, organisms reproduce asexually (Littleford 1939).

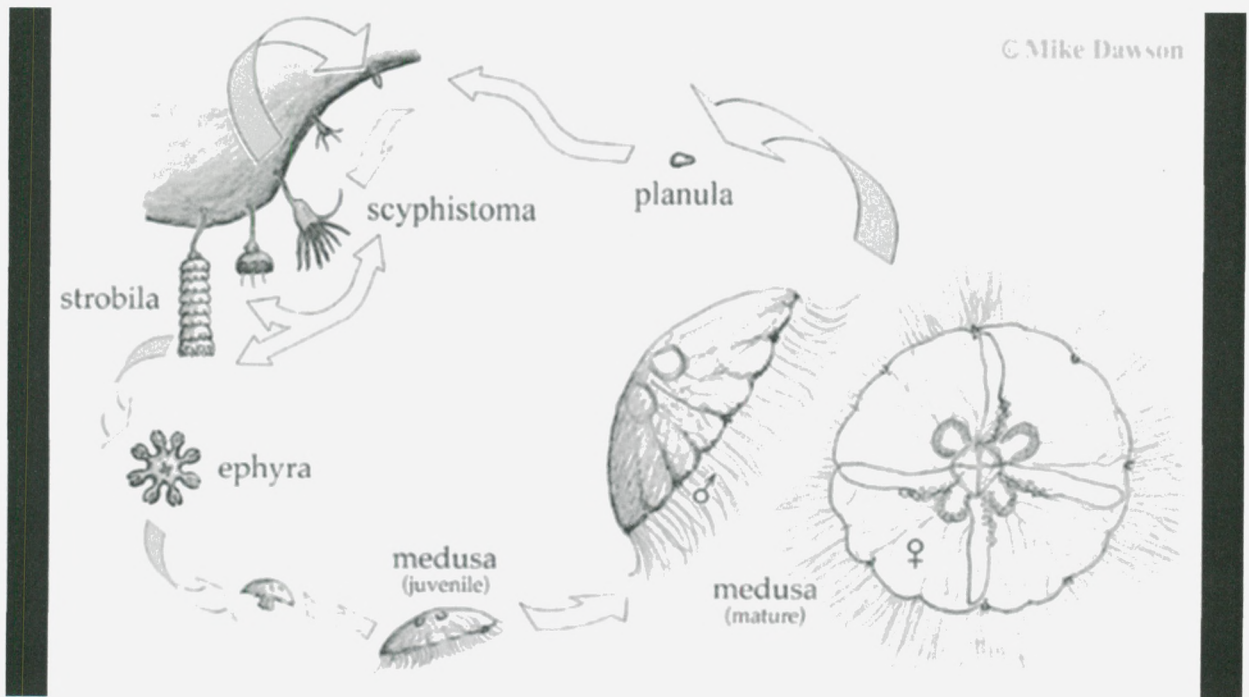


Figure 3: Scyphozoan Life Cycle. This image is by Michael Dawson (Dawson et al. 2005).

Sexual Reproduction

In sexual reproduction, the color of the gonads in a medusa determines the sex of the organism. Male gonads appear bright pink, whereas female gonads appear a greyish-brown or a yellowish-brown in color. When immature, the female sex cells, or ova, are transparent, with a distinct nucleus. When mature, the egg appears yellow due to the presence of a yolk rich cytoplasm needed for nutrition. The average size of a mature egg is 0.15 mm in diameter, but can range from 0.07-0.19 mm in diameter. The male sex cells, or spermatozoa, develop inside sacs of varying size and shape. When mature, the eggs are released from the gonads and enter into the gastrovascular cavity, where fertilization may occur, or exit the organism via the mouth and fertilization occurs

externally (Littleford 1939). Up to 40,000 eggs are released daily in the water per medusa (Purcell 2012).

Planula Stage

Within six hours following fertilization, a membrane develops around the egg and the egg begins to lengthen at one end resembling the shape of a pear (Littleford 1939). Planulae appear similar in shape to other *Chrysaora* species, such as *C. lactea* (Morandini et al. 2004). Within three hours, there are three rounds of cellular division, resulting in eight cells of equal size. Over a 10-12 hour time period, additional cleavage events take place and a blastula forms. The fertilization membrane is lost during this period of division. The blastula, which is a ball of cells, then begins to invaginate and develops into a round specimen, a planula (Littleford 1939).

A planula contains two primary germ layers: endoderm and ectoderm. The initial round shape of a planula becomes pear-shaped within about two to three hours, where the wide end faces anterior and the narrow end faces posterior. Within 24 hours, a planula will begin to rotate due to the presence of cilia located along the outside of the organism. The planula then swims freely and quickly, in any direction. However, the cilia only provide short distance movement in the water. The water current contributes to long-distance traveling for a planula (Littleford 1939).

The planula stage is short-lived, only lasting about three to five days, where ultimately the planula will settle down and its anterior, wider end will attach itself to a substrate, generally a hard surface. When this occurs, the organism is considered a scyphistoma, or a polyp (Littleford 1939).

Polyp stage

A polyp is divided into three regions: a base, stalk and cup-like oral end. The base is at the basal end, and is the portion that attaches to a substrate. The stalk is the length of the organism. The oral end is at the apical end of the organism. A pedal disc, which is a small disc-like structure that contains a sticky adhesive substance, forms at the base during attachment, and binds the organism to a substrate. Polyps have the ability to move, by making a new attachment with a substrate. Therefore, some polyps (older polyps) may have more than one pedal disc, as a new pedal disc is formed every time it makes an attachment with a substrate (Littleford 1939).

Within approximately one to three hours following attachment, a mouth develops at the center of the oral end. Initially, the mouth exists as a tiny slit. The mouth then grows wider, and indentations develop. The end result is a cruciform or cross-shaped mouth. Within five days following attachment, four primary tentacles develop, one at a time, around the mouth at the oral end. Between the four primary tentacles arise four indentations that project into the gastrovascular cavity. By day 10 following attachment, four secondary tentacles develop, each evenly distributed between two primary tentacles. By day 15, eight tertiary tentacles develop (Littleford 1939).

A polyp's body wall contains the same two primary germ layers observed in planulae: endoderm and ectoderm. Mesoglea also exists between the two primary germ layers, however the thickness varies. Initially, the mesoglea is very thin, but once the organism develops its primary and secondary tentacles, the mesoglea becomes much thicker and contains muscle fibers and other cellular structures (Littleford 1939).

Nematocysts develop on the external surface of all tentacles, which are stinging cells the organism will use to capture their prey and also to ward off predators. Two types of nematocysts are present, atrichous isorhizas and heterotrichous euryteles (Calder 1972b).

A polyp is continually growing in size during and after development. If feeding conditions are well, a polyp will obtain full development and size within three weeks (Calder 1972a). Polyps appear as “pink buttons” when observed with the naked eye. On average, the stalk will reach up to 1.5 mm in height, sometimes up to 3.5 mm in height, and reaches 0.6 mm in diameter, with tentacles of up to 6 mm in length. The body size of a polyp depends upon the degree of contraction of a polyp (Littleford 1939).

Polyps can live year-round; however, they thrive from spring months (late April or May) throughout the summer months (Calder 1972). When compared to other *Chrysaora* polyps, *C. hysoscella*, *C. lactea*, and *C. melanaster* contain as many or fewer number of tentacles. Appearance varies where *C. quinquecirrha* appear pink and other *Chrysaora* species appear whitish in color. *Chrysaora quinquecirrha* and *C. lactea* have longer stalk lengths when compared to *C. hysoscella* and *C. melanaster*, however, *C. quinquecirrha* is generally taller than *C. lactea*. *Chrysaora lactea* on average is 0.4-0.6 mm in height, but can reach up to 1.2 mm in height, and usually ranges 0.2-0.36 mm in diameter. Even though both *C. quinquecirrha* and *C. lactea* develop four primary tentacles, initially, *C. lactea* develops only two primary tentacles. It usually takes much longer to generate all four primary tentacles, where it can take up to 75 days. Secondary and tertiary tentacles will develop, 16 tentacles total, similar to *C. quinquecirrha*, however this number can vary from 12-21 (Morandini et al. 2004).

Settlement

Chrysaora quinquecirrha polyps have been observed on both the upper surface and underside of a hard substrate, but are generally found on the underside of hard surfaces, such as oyster shells. It is hypothesized that polyps select the underside purposefully in order to avoid the natural settling of sediments on the upper surface of the substrate. An accumulation of sediment on and around a polyp may lead to death. Therefore, selectively attaching to the underside can attribute to the survival of a polyp. Also, attaching to the underside of a surface is beneficial in hiding from predators. Polyps are less easily seen on the underside than on the upper surfaces. Another contributing factor could be light. It may be possible that polyps survive better in the absence of direct light, which could be why a polyp would attach to a dark underside of a surface (Cargo and Schultz 1966). Attaching to the underside of a substrate is common to other cnidarian polyps as well, which has been observed in *Aurelia* (Fraser 1962).

In the Chesapeake Bay, *C. quinquecirrha* polyps have been observed on substrates up to a depth of 11 meters in the water. Even though polyps are most commonly found on oyster shells or the underside of a hard substrate, they seem to attach to any hard surface available, both natural and man-made substrates (Cargo and Schultz 1966; Hoover and Purcell 2009). Natural substrates include rocks, shells, polychaete tubes, ascidians, algae, and bryozoans (Hoover and Purcell 2009). Man-made substrates are usually preferred over natural substrates and include docks and/or bulkheads made of plastics, ceramics, and other dock-building materials (Holst and Jarms 2007). In a study where six common dock-building materials were tested to see if *Aurelia* polyps would

attach, the polyps attached to all materials, but preferred plastics like polystyrenes and polyethylenes over rubber and treated wood (Hoover and Purcell 2009).

Asexual Reproduction

Chrysaora quinquecirrha can reproduce asexually three ways: strobilation, cyst production, and by changing polyp position via the use of a stolon (Littleford 1939). If environmental conditions are favorable, a polyp will undergo strobilation or transverse segmentation (Littleford 1939). Conditions are favorable when temperature, food availability and light levels are high, and salinity levels are relatively low (Purcell et al. 1999). A polyp will horizontally divide into different segments, and will ultimately divide into two separate organisms within a time span of four to five days (Littleford 1939).

During this time, a polyp prepares for strobilation by developing a number of horizontal shallow grooves in the body wall. The grooves deepen and tentacles are reabsorbed into the body. Eventually the grooves divide the body into separate horizontal sections, all attached to one another by a central cord. Typically five or six segments are created, but the number can vary. Each section then develops eight indentations, which deepen and divide each section into eight lobes. Each lobe then begins to split, and at the apex of the split a rhopalium (sensory organ) develops. At this point, the organism is now considered a strobila. Each segment begins to pulsate and then detaches from the central cord and is released into the water. Segment detachment usually takes about 10 to 16 hours (Littleford 1939).

The end result is a free-swimming ephyra (Littleford 1939). The portion of the original polyp that remains is a small piece of the proximal end, and is fully capable of regenerating. The polyp regains its normal size and shape and is then able to undergo asexual reproduction again (Calder 1974).

It is possible to tell if a polyp has recently undergone strobilation as there are three morphological differences. First, the mouth opening appears as a tiny slit. Second, the length of the body is short and broad. Third, the shape of the mouth resembles a crater, or a small round depression. However, polyps are resilient and will retain their normal physical appearance within five to seven days post-strobilation (Littleford 1939).

Temperature plays a role in strobilation, which has been observed both in the field and in the lab. In the Chesapeake Bay, strobilation generally begins in April as water warms to 17°C. Strobilation does not occur between November and March as the water temperature is too cold (Calder 1974). When temperature was manipulated in the lab, strobilation was induced when water temperatures were increased (Cones 1969; Loeb 1972). Other factors also contribute to strobilation, such as feeding supply, like plankton blooms (Thiel 1962). Optimal salinity, dissolved oxygen, light, chemicals, and pH are also factors for strobilation (Spangenberg 1968).

Each polyp can produce more than one ephyra per strobilation event and it is possible for a polyp to undergo strobilation more than once in a lifetime. Previous studies demonstrate varying results of numbers of ephyra produced. Cargo and Schultz (1966) reported that each polyp can produce three to nine ephyra, and on average, a polyp will produce five ephyra. Calder (1974) reported that polyps can each produce between one to 16 ephyra, but also focused on seasonal rates and discovered that higher numbers

were produced in spring when compared to summer months. Loeb (1972) reported five to 20 ephyra produced per polyp. In terms of strobilation event rates, in a 12 month study of collecting polyps in Sarah Creek just north of the Chesapeake Bay, Calder (1974) observed polyps in the lab for 24 days and reported that of 48 polyps, 22 polyps underwent strobilation once, 11 underwent strobilation twice, four strobilated three times, and four strobilated four times.

Strobilation rates have been observed with multiple peaks throughout each year. There are many explanations for the peaks. Early peaks are due to strobilation of polyps that survived the cold, winter season. Second and larger peaks are due to excystment of polyps from podocysts and their ensuing strobilation. Later peaks are due to repeated strobilation in polyps (Cargo and Schultz 1967). Strobilation peaks also correlate with increasing tidal amplitudes, as seen by Calder (1974) in Sarah Creek, which suggests the alignment of the sun and moon influence strobilation rate.

When conditions are unfavorable for *C. quinquecirrha* polyps to grow or strobilate, such as when water temperatures decrease, polyps have the ability to form cysts (Teheou-Tai-Chuin 1930). The organism is then able to go dormant until environmental conditions improve, which is beneficial to the survival of the organism. Cysts are also of value because they can protect against predators, such as nudibranchs (Arai 2009). In the Chesapeake Bay, *Cratena* species have been documented to feed on polyps, but not podocysts, showing that podocysts protect against predation (Cargo and Schultz 1967). Cysts can also increase population numbers, and they contribute to short-term survival of the organism during low food supplies (Arai 2009).

A cyst can be formed from a whole polyp, a planula as it settles on a substrate, or by a portion of a stolon from a polyp coming in direct contact with a substrate (Arai 2009). Cysts produced by planula are called planulocysts and polyps produce podocysts. Podocysts are irregular disc-like structures with a round depression on the superior aspect, resembling a dome-shape (Holst and Jarms 2007; Blanquet 1972). They appear white on the inside, with a reddish-brown coating on the outside (Blanquet 1972). Podocysts are approximately 0.35-0.45 mm in diameter and 0.08-0.1 mm in height (Littleford 1939; Morandini et al. 2004). Small pieces of epidermal tissue and mesenchymal cells from a polyp's pedal disc spread into two or more parts and separate from the pedal disc. A thin leathery cuticle composed of a chitin-protein complex develops around the separated tissue and then is called a podocyst (Blanquet 1972; Cargo and Schultz 1966). Another way a polyp can develop a podocyst is when a stolon grows laterally from the stalk of a polyp and then moves down until it eventually makes a connection with a substrate (Cargo and Schultz 1966).

Chitin contributes to the reddish-brown outer coloring of a podocyst and ranges from 9-13 μm in thickness. The cuticle is composed of individual concentric lamellae, or circular layers of chitin, which resemble a tree-trunk with its rings. Each lamella contains many microfibers that pack themselves together in sheets (Blanquet 1972). The center of a podocyst appears lighter in color than the outer region, as the innermost region is much thinner in comparison (Cargo and Schultz 1966). The cuticle provides an excellent barrier to the podocyst and provides protection against chemical attacks because it is resistant to enzymatic degradation. In addition, if chitin were to break down, it has the ability to develop a new cuticle (Blanquet 1972).

Once a podocyst is formed, a polyp forms a stolon and is then able to move to another section of the substrate and leaves the podocyst behind. At the new site, podocyst formation can occur again and the polyp can move to a new location. Therefore, one polyp has the ability to develop multiple podocysts (Arai 2009). For example, in the lab, it was reported that a single polyp in culture produced 52 podocysts and six polyps in less than three months (Cargo and Schultz 1966).

Even though individual polyps do not have the ability to produce more polyps directly, more than one polyp can be produced as one polyp can produce multiple cysts, where each cyst can contribute to a new polyp (Arai 2009). When conditions are again favorable, such as food is readily available and water temperatures increase, podocysts can develop from polyps in as little as a few weeks (Cargo and Schultz 1966). More polyps can increase the production of ephyrae, which then leads to more medusae (Arai 2009). Therefore, cyst production can be a major contributing factor to jellyfish blooms.

Food availability plays a role in podocyst production. *Chrysaora quinquecirrha* polyps without food for long periods of time will result in a decrease in size and will not produce cysts. The polyp may not receive the required nutrients to provide energy to develop a cyst. Polyps produce cysts when food is readily available (Littleford 1939). Cells in the central storage zone of a podocyst contain yolk made of carbohydrates, proteins, and lipid reserves. These reserves are used when conditions worsen and food is unavailable during the winter months. It is unclear how long a polyp or podocyst can survive for in its natural environment. However, previous studies indicate that half of the DNA, one-third of the protein, and one-fifth of the lipid reserves within a podocyst are lost within one year of formation (Black 1981). These results suggest that a podocyst is

beneficial for survival during short unfavorable seasonal changes, such as when water temperatures drop and food becomes scarce for a polyp. A podocyst would most likely not survive more than two years, as most of its reserves would be depleted.

Temperature also plays a role in cyst production. When water temperatures decrease by 2-4°C, podocyst production is seen (Cargo and Schultz 1967). When water temperatures rise to 15-18°C, the internal tissues of a cyst will develop into a small polyp with four tentacles (Cargo 1974). This new polyp will then develop into a full-grown polyp and is then able to produce more podocysts or strobilate into an ephyra (Cargo and Rabenold 1980). Polyps can also produce cysts in low oxygenated water. When oxygen was removed from water with hydrogen sulphide, polyps produced cysts (Cargo and Schultz 1966).

Ephyra stage

The product from strobilation of a polyp is an ephyra, which is initially red or pink in color, round in shape, with radiating projections surrounding the outer perimeter of the organism. Throughout development, an ephyra changes color, to a whitish or translucent appearance (Littleford 1939).

As seen in Figure 4, there are six distinct stages from the development of an ephyra (early medusa) to an adult medusa, or jellyfish (Calder 1972a). Ephyra growth can occur very quickly, where the organism can increase in size ten times within as little as a few days (Littleford 1939).

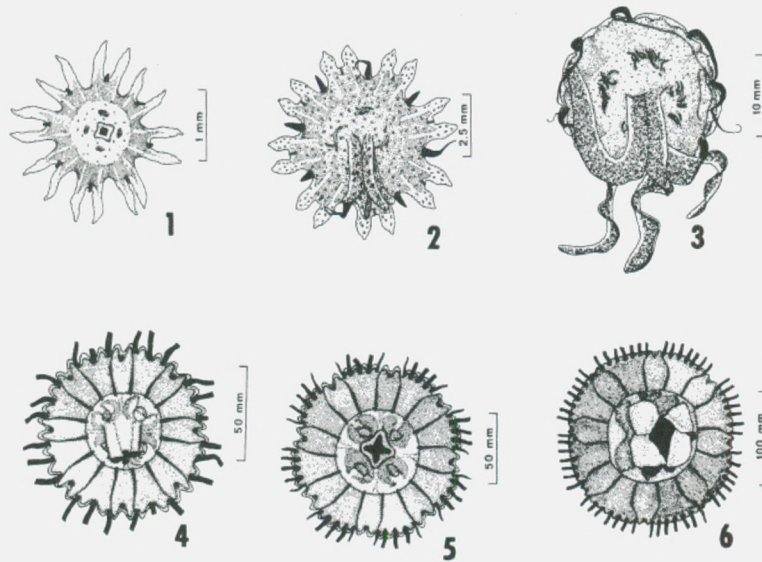


Figure 4: Six stages of development from ephyrae to medusa. This image was taken from Calder (1972a).

During stage one, the newly developed ephyra via strobilation has a round body, with eight pairs of lappets, which are thin flap-like structures, found projecting off the outer perimeter of the round body (Calder 1972a; Littleford 1939). The early ephyra remains close to the bottom surface of the water and swims with the subumbrellar surface facing upwards (Littleford 1939). The organism is generally 0.84 mm in diameter and can grow up to 2.0-3.5 mm wide when measured from lappet-tip to lappet-tip (Littleford 1939; Calder 1972a). Between each lappet pair is a rhopalia, which is a small sensory structure. In total, there are eight rhopalia. Rhopalar clefts, which are u-shaped or v-shaped gaps, are wide between lappet-tips and narrow at the base of the lappet pairs. At this stage, the mesoglea is a very thin layer and the gastrovascular cavity is divided into 16 pouches (Calder 1972a). The manubrium, which is carried in an upward position, is found in the center of the round body, is short and square in shape, only about 0.23 mm in length (Calder 1972a; Littleford 1939). Tentacles are not present during this stage (Calder 1972a). Nematocysts exist within three different sized capsules. Nematocysts

are found covering the surface of the exumbrella (the top of the organism) as well as the middle of each lappet (Littleford 1939).

During stage two, eight primary tentacles develop around the rim of the body, each between the lappet pairs. Tentacles will develop within one to three days upon strobilation, and all develop at the same time at equal rates. Oral arms develop at each corner of the manubrium, one at a time. Lappets widen and begin to fold downward (Calder 1972a). The round body starts to grow outward, filling in the clefts that divide the lappets (Littleford 1939).

During stage three, which occurs 10-14 days following strobilation, the organism loses the appearance of an ephyra and starts to resemble a medusa. The lappets continue folding downward, oral arms are more developed and can move freely on their own, and the manubrium grows larger (Calder 1972a). Eventually, the manubrium increases in length enough that it is longer than the bell diameter. When this occurs, the bell becomes inverted and the manubrium hangs downward from the center of the subumbrella. Four gastric cirri, hollow, finger-like projections that bear nematocysts, develop at the interradii of the manubrium on the subumbrellar surface. More gastric cirri are produced as development continues (Littleford 1939).

During stage four, which occurs within 25 days after strobilation, secondary tentacles develop between primary tentacles and rhopalia. The 8 pairs (16 total) of lappets divide into two, resulting in 32 individual tongue-shaped lappets. Oral arms lengthen and become ribbon-like. Gonads develop within four gastric pouches (Calder 1972a). *Chrysaora quinquecirrha* is now dioecious, where the species can either contain male or female gonads and are now sexually mature (Calder 1974).

During stage five, each tertiary tentacle, 16 total, develops lateral to a secondary tentacle. Each lappet adjacent to a tertiary tentacle divides into two, resulting in 48 lappets total. There are now 40 tentacles in total present. This stage is also referred to as the *Dactylometra* stage (Calder 1972a).

During stage six, the medusa is fully developed, divided into octants, or eight regions. Within each octant are seven or more tentacles and eight or more lappets. The adult medusa contains at least 56 tentacles and 64 lappets (Calder 1972a).

Medusa stage

Adult medusae grow rapidly, where the bell diameter can grow as much as 5 mm per day (Calder 1972a). They are “free-swimming”, where they swim in a constant, vertical motion. They move by both passive drifting and contractions of the body. These body contractions are due to pulsations of the swimming bell, which are produced by nerve centers found on the outside of the bell (Costello et al. 1998). As the medusa develops, they can begin to release their eggs or sperm daily when they are as small as 100 mm in diameter (Calder 1972a; Purcell 2012). Up to 40,000 eggs are generally shed daily in the water per medusa (Purcell 2012). Medusa mature within two months, generally around late June or July (Calder 1972b). Medusa population numbers are generally high in the summer, where each polyp will produce about 45 medusae every year (Purcell 2012). Medusae are short-lived, only surviving a few months. Once their gonads have been spent, they begin to die, generally in September (Calder 1972b). In addition, the colder water temperature in the winter slows the organism’s ability to swim and leads to starvation (Sexton et al. 2010).

Seasonal Distribution

Chrysaora quinquecirrha survive year-round, but each stage exists at certain seasons. Polyps are generally found in abundance in the spring, late summer, and fall months and are fewer in the winter and mid-summer months. In a 12-month experiment from March 1972 to February 1973, strobilation was first observed in April, with the highest rates in May and June, and then slowly declined in the late summer months. No strobilation was observed after October. Strobilation correlated with tidal amplitudes, where the highest peaks of strobilation corresponded with increasing tidal amplitudes (Calder 1974). In the lab, polyps cultured for four years at 20-25°C demonstrated strobilation each summer, then formed podocysts in the fall and either broke down or released polyps within a few months (Littleford 1939). In the Chesapeake Bay, podocysts that were produced in August produced polyps and strobilated the following spring when water temperatures increased (Cargo and Schultz 1967; Cargo and Rabenold 1980). Many ephyrae are released in the spring (late April and May) due to strobilation and throughout the summer months. Ephyrae develop into adult medusae within a couple months each summer. Medusae survive for only a few months each year. They generally live until September, but can survive as late as November. Medusae generally live in creeks weeks before that appear in bays or major rivers (Calder 1972).

Feeding Behavior

Chrysaora quinquecirrha polyps demonstrate a specific feeding behavior in response to low concentrations of most amino acids and many small peptides. Polyps capture their prey using their nematocysts. The tentacles contract and bend towards the

mouth. The mouth opens and the tentacles insert into the mouth and gastrovascular cavity, thereby inserting the food. Tentacles respond to most amino acids and small peptides, indicating there may be feeding reflex receptors on tentacles (Loeb and Blanquet 1973).

Chrysaora quinquecirrha are carnivores. Adult medusae mainly feed on small crustaceans, such as copepods and ctenophores, like comb jellies. During the ephyra stage, ephyrae prefer the ctenophore, *Mnemiopsis leidyi*, comb jelly larvae. Ephyrae also like to eat copepods, rotifers, and zooplankton (Olesen et al. 1996). Polyps generally eat anything that is available to them, such as copepods, comb jelly larvae, brine shrimp, and protozoans. Due to the diverse selection of prey, this organism rarely starves. When conditions are good and food intake increases, polyp population numbers do not increase, but instead somehow triggers strobilation and produces ephyrae (Purcell et al. 1999).

Mutualism

Chrysaora quinquecirrha and oysters demonstrate mutualism, where *Chrysaora* polyps use oyster shells as its substrate and when attached, *Chrysaora* provides protection against predators for a developing larval oyster. One major predator of oyster larvae is comb jellies. If a comb jelly comes into contact with the oyster shell, *Chrysaora* will prey on it. Because of this, *Chrysaora* is able to control comb jelly populations in the summers. Wherever *Chrysaora* polyps are vastly present in the bay, comb jelly populations are decreased (Purcell 2012).

Chapter Two: PCR Amplification and DNA Sequence Analysis of the 45S Ribosomal DNA Cassette of *Chrysaora quinquecirrha*

Introduction

Chrysaora quinquecirrha, most commonly known as the “sea nettle” is an aquatic species that belongs to the phylum Cnidaria and class Scyphozoa. Sea nettles are found in waters of low salinity, mainly in estuaries. They are found off the coast of the Atlantic, Indian, and Western Pacific Oceans (Mayer 1910). In the United States, they are found in the Chesapeake Bay, which is the largest estuary in the United States bordering Maryland and Virginia. They have more recently been found in the Barnegat Bay and Navesink River of New Jersey.

Cnidarians exhibit a diploblastic, radial symmetric body plan, which contains a gastrovascular cavity and a single opening that acts as both a mouth and anus. The life cycle is very complex and two body forms exist in an alternation of generations: a sessile polyp stage and a free-swimming adult medusa (jellyfish) stage. Both forms have cnidocytes, which are specialized cells that help defend predators and capture prey by injecting poison (Calder 1972b). A sea nettle’s sting is painful and can cause a rash on the skin of those impacted. However, the sting is not lethal and pain does not last for long periods of time. More recently, sea nettles have been of concern to humans due to overpopulation. It is possible that humans are impacting on increase in population size of sea nettles, by introducing chemicals via runoff into the Bay from waste products and fertilizers, and also providing additional substrates for polyps, such as floating docks and bulkheads (Hoover and Purcell 2009).

Customarily, classification of cnidarians has been based mainly on morphological characteristics. Evolutionary and phylogenetic relationships have been revised a number of times using morphology; therefore many hypotheses exist and are dependent upon what factors are used for classification. Only more recently with the advancement of molecular tools has phylogenetics begun to consider molecular analyses.

For cnidarians, there is beginning to be a focus on mitochondrial and ribosomal DNA. Mitochondrial DNA (mtDNA) can be used as a marker to identify a species. It can also help to indicate evolutionary and phylogenetic relationships among different species and even populations of species (Galtier et al. 2009). For example, mtDNA shape has been used to draw phylogenetic relationships among the four major classes of cnidarians, where class Anthozoa has circular mtDNA and Scyphozoa, Cubozoa, and Hydrozoa all have linear mtDNA (Bridge et al. 1992). This molecular evidence supports the notion that Scyphozoans, Cubozoans, and Hydrozoans are most closely related to one another in comparison to Anthozoans. This evidence is consistent with the cnidarian life-cycle hypothesis that Anthozoans who have a polyp-only life cycle originated first and the three other classes, who have a polyp-medusa life cycle, evolved from class Anthozoa (Bridge et al. 1995).

Ribosomal DNA (rDNA) has also been considered when studying cnidarian relationships. rDNA is the region of a genome coding for the RNA component of ribosomes. In eukaryotes, this DNA is generally found in tandem repeats, and is referred to as the 45S rDNA cassette. rDNA can have up to 10,000 copy numbers (Schlotterer 1998). Each copy is separated by a spacer, which links each copy together. The genes within the 45S rDNA cassette are referred to as dosage repetition genes, as there are

many copies of each gene. Generally, a cell will have more ribosomal genes than the minimum required for a cell to maintain viability. The high copy number allows for higher efficiency of ribosomal DNA functioning to fulfill the requirements of a cell, as having only one copy would produce much less product in the same amount of time (Long and David 1980).

The cassette varies in copy number and in length among different species. In humans, one copy is approximately 13,300 bp in length with 400 copies (Zentner et al. 2011). In yeast and *Drosophila*, the cassette is much shorter, approximately 8,000 bp in length. Birds generally have a larger cassette of 10,500 bp and mammals usually about 13,000 bp (Long and David 1980). In the moon jelly, *Aurelia*, one unit is 7,731 bp long (Ki et al. 2009).

Each copy of the 45S rDNA cassette is composed of genes coding for the 18S, 5.8S, and 28S rDNA, which are necessary for ribosomal functioning. The 18S gene codes for the small subunit of a ribosome and the 28S gene codes for the large subunit of a ribosome (Hillis and Dixon 1991). The 5.8S gene is also transcribed and then is thought to be involved with ribosome translocation (Elela and Nazar 1997). These genes are encoded as a single transcription unit. Between these genes are spacer regions. There are two non-coding internal transcribed spacer (ITS) regions: ITS1 and ITS2. ITS1 is located between the 18S and 5.8S gene and ITS2 is located between the 5.8S and 28S gene. An intergenic spacer (IGS), which contains a non-transcribed spacer (NTS) region and an external transcribed (ETS) region, connects each copy of the cassette together (Hillis and Dixon 1991). The IGS region contains an enhancer and promoter region (Zentner et al. 2011). The ITS regions are thought to play a role in the maturation of

rDNA genes (Baldwin et al. 1995). When fragments of ITS1 were deleted, it led to inhibiting the maturation of the small and large ribosomal subunits (Musters et al. 1990). Additionally, deletions of fragments of ITS2 led to a reduction or complete inhibition of the large ribosomal subunit (van der Sande et al. 1992). Each copy is organized in a head-to-tail arrangement: 18S, ITS1, 5.8S, ITS2, 28S, IGS as seen in Figure 5 (Long and David 1980).

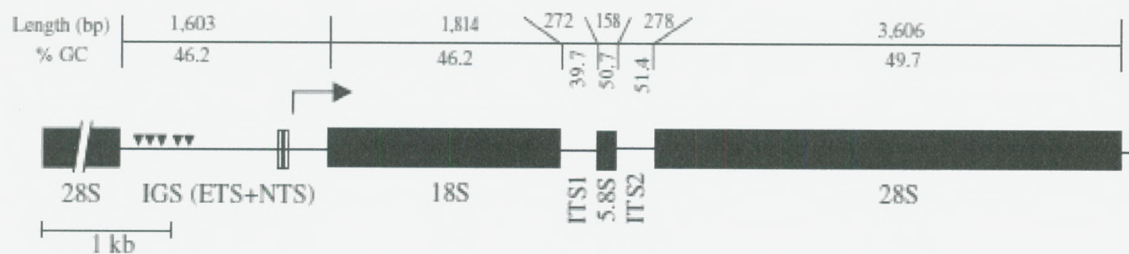


Figure 5: Schematic of 45S rDNA cassette in the moon jelly, *Aurelia*. This image was taken from Ki et al. (2009).

Each region of the cassette displays different amounts of variability among species, which allows for comparison. The coding genes have a very slow evolutionary rate and are highly conserved among related species (Hillis and Dixon 1991). Spacer regions, such as ITS1 and ITS2, have a high nucleotide substitution rate which leads to both variation between and within species (Hillis and Dixon 1991). Each copy of the 45S rDNA cassette within an organism is nearly identical to one another due to unequal crossing over and gene conversion referred to as concerted evolution (Ambrose and Crease 2011). The mechanism of concerted evolution explains why ITS regions demonstrate lower variation within species and higher variation between species (Aguilar et al. 1999). For these reasons, the 45S rDNA cassette is useful for species identification and phylogenetic studies.

18S rDNA was analyzed in 19 different cnidarian species (not including *Chrysaora quinquecirrha*). Of the 19 species, 3 belonged to class Scyphozoa. 18S sequence data was analyzed along with morphological characters, mitochondrial 16S rDNA, and mitochondrial gene structure to predict phylogenetic relationships. Results support the hypothesis that states the polyp-medusa life cycle originated from the polyp-only life cycle (Bridge et al. 1995).

Other recent studies have investigated the 18S and 28S sequences of various cnidarians, including all classes. However, most studies only sequence and analyze partial 18S or 28S sequences. In addition, the studies are incomplete in that the number of different species is limited due to sample availability. With limited sequence data available, it is difficult to draw conclusive phylogenetic relationships. The following *Chrysaora* species have been studied in phylogenetic analyses: *C. fuscescens*, *C. lactea*, and *C. melanaster* (Collins et al. 2006; Bayha et al. 2010). *Chrysaora quinquecirrha* 18S and 28S rDNA has not been analyzed in any published phylogenetic or molecular study as of yet.

ITS regions can be a useful marker if a high number of reference sequences are available (Machida and Knowlton 2012). However, very few partial ITS1 sequences and nearly no ITS2 sequences have been identified in cnidarians (Croce et al. 2006). In a study where the ITS1 region was identified and sequenced for 599 marine species, of which 59 were cnidarians, it was concluded that invertebrates generally have shorter ITS1 sequences and a lower GC% content (117 - 1613 bp and 35.8% to 71.3%) when compared to vertebrates (318 to 2318 bp and 56.8% to 78%). Specifically for cnidarians, the average length of the ITS1 region was 253.9 bp with an average GC% content of

45.6% (Chow et al. 2009). Based on this study, GC% content and ITS1 length are directly correlated.

In the same Pelagiidae family as *C. quinquecirrha* is *Aurelia*. *Aurelia* is a well-studied widely distributed species and much of its genomics have been investigated. Recently, the entire mitochondrial DNA sequence was determined and identified for both *Aurelia* and *C. quinquecirrha* (Hwang et al. 2013a, 2013b). It turns out that the mitochondrial DNA of *C. quinquecirrha* is very similar to *Aurelia*. *Aurelia* is currently the only cnidarian completely sequenced for the 45S rDNA cassette (Ki et al. 2009). Figure 5 illustrates the organization and size of the 45S rDNA cassette in *Aurelia*: the 18S rDNA gene is 1814 bp, ITS1 is 272 bp, 5.8S rDNA gene 158 bp, ITS2 is 278 bp, and 28S rDNA gene is 3606 bp long. Ki et al. were successful in determining the 45S rDNA cassette because each segment of the 45S rDNA cassette is relatively short, high in copy number, and therefore easy to amplify and sequence. Because *C. quinquecirrha* and *Aurelia* are very similar species, where they belong in the same cnidarian family and have similar genomics, it would be safe to assume that the 45S rDNA cassette in *C. quinquecirrha* will be very similar to that of *Aurelia*'s as well.

In order to reduce uncertainty in relationships among cnidarians, additional evidence needs to be compiled and analyzed for *C. quinquecirrha*. This will allow for a better understanding of evolutionary and phylogenetic relationships. This can be done by collecting and analyzing all ribosomal molecular data from *C. quinquecirrha*.

The aim of this research is to determine the nucleotide sequence of the 45S rDNA cassette in *C. quinquecirrha* by performing a PCR amplification and DNA sequence analysis, similar to that of Ki et al. (2009) for *Aurelia*, to increase available data for

phylogenetic analyses. In this study, specific primer sets will be designed to PCR amplify short fragments of the 45S rDNA cassette. Successful amplification will be confirmed via gel electrophoresis. DNA samples will be sequenced and resulting electropherograms will be viewed and edited for accuracy. Overlapping sequences from each primer reaction will then be assembled into a consensus sequence, which can then be analyzed to understand the relationship to other cnidarians as well as other organisms.

This research will be useful for phylogenetic studies as molecular data adds another element when classifying and identifying organisms. This information will help support current cnidarian evolutionary and phylogenetic hypotheses. With molecular data from the 45S rDNA cassette, *C. quinquecirrha* can be compared to other cnidarians to determine its relatedness among species. Also, individual sea nettle populations can be compared to determine relationships to one another.

Materials and Methods

In silico Analysis and Primer Design

18S and 28S ribosomal DNA sequences of the 45S ribosomal DNA cassette were identified using a BLAST search (<http://blast.ncbi.nlm.nih.gov>) with known sequences from other cnidarians: *Chrysaora fuscescens* (HM194815.1, HM194868.1), *Chrysaora melanaster* (HM194811.1, AY920780.1), *Chrysaora lactea* (HM194810.1, HM194863.1), *Chrysaora quinquecirrha* (HM015266.1), *Chrysaora* sp. (AY920769.1, AY920779.1), *Aurelia* sp. (EU276014.1), and *Cyanea capillata* (HM194820.1, HM194873.1). Sequences were aligned using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) (Fig. 6-7).

Relative positions of previously designed primers were identified using a BLAST search against other cnidarians (Table 1, see Results Fig. 8). Primers were analyzed and checked for hairpins, self-dimers and hetero-dimers using Oligo Analyzer software (<http://www.idtdna.com/analyzer/applications/oligoanalyzer/>) at Integrated DNA Technologies (www.idtDNA.com).

Primer sets were then selected by matching for homology against published sequences through a BLAST search and Clustal Alignment (Fig. 6-7). Primers were ordered from IDT and resuspended in sterile deionized water according to manufacturer's instructions. For each primer, a 10 μ M stock was made in sterile deionized water for PCR reactions and stored at -20C.

Primer Name	Nucleotide Sequence (5' \rightarrow 3')	Length (nt)	% GC content	Tm ($^{\circ}$ C)	Location
P1 (F)	CTCGTAGTTGGATTTCGGGA	20	50.0	60.4	18S
P2 (R)	AACTAAGAACGGCCATGCAC	20	50.0	60.4	18S
P3 (F)	AACCTGGTTGATCCTGCCAGT	21	52.4	62.6	18S
P4 (R)	GATCCTTCTGCAGGTTACCTAC	23	52.2	64.6	18S
P5 (F)	ACCCGCTGAATTTAAGCATA	20	40.0	56.3	28S
P6 (R)	GCTTTGGGCTGCAAAGCAACCCACTC	26	57.7	73.9	28S
P7 (F)	GAACRGCTCAAGCTTAAATCT	22	36.4	58.9	28S
P8 (R)	GAAACTTCGGAGGGAACCAGCTAC	24	54.2	66.3	28S
P9 (R)	ACGAACGATTTGCACGTCAG	20	50.0	60.4	28S
P10 (R)	AACCAGCTACTAGRYGGTTTCGAT	23	43.5	62.8	28S
P11 (F)	GGTTTCCGTAGGTCTGCGGAAGGATC	26	57.7	71.5	ITS1
P12 (R)	CGCACGAGCCGAGTCCACCTTAGAAG	26	61.5	71.7	ITS1
P13 (F)	TAGGTGAACCTGCGGAAGGA	20	55	58.1	18S
P14 (R)	TATGCTTAAATTCAGCGGGTAG	22	40.9	52.7	28S

Table 2: Summary of Primers. Primers 3 and 4 (Medlin et al. 1988) were used by Bayha et al. (2010) to analyze phylogenetic relationships among 46 jellyfish species. Primers 5, 9, and 10 were designed by Matsumoto et al. (2003) by modifying universal primers (Scholin and Anderson 1994). Primers 7 and 8 were designed by Bayha et al. (2010). Primers 1, 2, 13, and 14 were designed in this study.

Relative positions of primer sets 1-3 were identified through a Clustal Alignment of known cnidarian 18S rDNA sequences (Fig. 6). Set 1 primers (1 and 2) were designed to amplify an internal fragment of the 18S rDNA and should yield a product of 665 bp. Set 2 primers (2 and 3) were used to amplify the first 1302 bases of the 18S rDNA sequence. Set 3 primers (1 and 4) were used to amplify the 3' end of the 18S rDNA sequence and should yield a product of 1171 bp. A summary of primer sets 1-3 is listed in Table 3.

Chrysaora_lactea	-----AGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT	39
Chrysaora_sp	-ACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT	59
Chrysaora_melanaster	-----AGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT	39
Chrysaora_fuscescens	-----AGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT	39
Cyanea_capillata	-----AGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT	39
Aurelia_sp.	TATCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT	60

Chrysaora_lactea	AAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATT	99
Chrysaora_sp	AAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATT	119
Chrysaora_melanaster	AAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATT	99
Chrysaora_fuscescens	AAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATT	99
Cyanea_capillata	AAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATT	99
Aurelia_sp.	AAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATT	120

Chrysaora_lactea	TGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAAAAGT	159
Chrysaora_sp	TGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAAAAGT	179
Chrysaora_melanaster	TGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAAAAGT	159
Chrysaora_fuscescens	TGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAAAAGT	159
Cyanea_capillata	TGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAAAAGT	159
Aurelia_sp.	TGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAAAAGT	180

Chrysaora_lactea	CCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTG---CTT-CGG	215
Chrysaora_sp	CCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTG---CCT-CGG	235
Chrysaora_melanaster	CCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTG---CTT-CGG	215
Chrysaora_fuscescens	CCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTG---CTT-CGG	216
Cyanea_capillata	CCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTG---CTT-CGG	219
Aurelia_sp.	CCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTG---CTT-CGG	239

Chrysaora_lactea	-TGCCCGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGA	274
Chrysaora_sp	-TGCCCGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGA	294
Chrysaora_melanaster	-TGCCCGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGA	274
Chrysaora_fuscescens	GCACCCGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGA	276
Cyanea_capillata	-CGCCCGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGA	278
Aurelia_sp.	GCACACGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGA	299
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Chrysaora_lactea	TGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATTGGT	334
Chrysaora_sp	TGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATTGGT	354
Chrysaora_melanaster	TGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATTGGT	334
Chrysaora_fuscescens	TGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATTGGT	336
Cyanea_capillata	TGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATTGGT	338
Aurelia_sp.	TGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATTGGT	359

Chrysaora_lactea	TACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC	394
Chrysaora_sp	TACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC	414
Chrysaora_melanaster	TACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC	394
Chrysaora_fuscescens	TACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC	396
Cyanea_capillata	TACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC	398
Aurelia_sp.	TACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC	419

Chrysaora_lactea	CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA	454
Chrysaora_sp	CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA	474
Chrysaora_melanaster	CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA	454
Chrysaora_fuscescens	CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA	456
Cyanea_capillata	CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA	458
Aurelia_sp.	CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA	479

Chrysaora_lactea	CAAGAAATAACAATCCGTGTCTATTCTTAGACGCGAAATTGGAATGAGTACAATTTAAA	514
Chrysaora_sp	CAAGAAATAACAATCCGTGTCTATTCTTAGACGCGAAATTGGAATGAGTACAATTTAAA	534
Chrysaora_melanaster	CAAGAAATAACAATCCGTGTCTATTCTTAGACGCGAAATTGGAATGAGTACAATTTAAA	514
Chrysaora_fuscescens	CAAGAAATAACAATCCGTGTCTATTCTTAGACGCGAAATTGGAATGAGTACAATTTAAA	516
Cyanea_capillata	CAAGAAATAACAATCCGTGTCTATTCTTAGACGCGAAATTGGAATGAGTACAATTTAAA	518
Aurelia_sp.	CAAGAAATAACAATCCGTGTCTATTCTTAGACGCGAAATTGGAATGAGTACAATTTAAA	539

Chrysaora_lactea	TCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATCCAGC	574
Chrysaora_sp	TCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATCCAGC	594
Chrysaora_melanaster	TCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATCCAGC	574
Chrysaora_fuscescens	TCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATCCAGC	576
Cyanea_capillata	TCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATCCAGC	578
Aurelia_sp.	TCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATCCAGC	599

Chrysaora_lactea	TCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGATGG	634
Chrysaora_sp	TCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGATGG	654
Chrysaora_melanaster	TCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGATGG	634
Chrysaora_fuscescens	TCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGATGG	636
Cyanea_capillata	TCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGATGG	638
Aurelia_sp.	TCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGATGG	659

Chrysaora_lactea	GCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTCTTCGCAAGACTGC	694
Chrysaora_sp	GCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTCTTCGCAAGACTGC	714
Chrysaora_melanaster	GCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTCTTCGCAAGACTGC	694
Chrysaora_fuscescens	GCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTCTTCGCAAGACTGC	696
Cyanea_capillata	GCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTCTTCGCAAGACTGC	698
Aurelia_sp.	GCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTCTTCGCAAGACTGC	719

Chrysaora_lactea	GTGTGCTCTTAGTTGAGTGTGCGTAGGATTGCGACGTTTACTTTGAAAAAATTAGAGTG	754
Chrysaora_sp	GTGTGCTCTTAATTGAGTGTGCGTAGGATTGCGACGTTTACTTTGAAAAAATTAGAGTG	774
Chrysaora_melanaster	GTGTGCCCTTAATTGAGTGTGCGTAGGATTGCGACGTTTACTTTGAAAAAATTAGAGTG	754
Chrysaora_fuscescens	GTGTGCCCTTAATTGAGTGTGCGTAGGATTGCGACGTTTACTTTGAAAAAATTAGAGTG	756
Cyanea_capillata	GTGTGCCCTTAATTGAGTGTGCGTAGGATTGCGACGTTTACTTTGAAAAAATTAGAGTG	758
Aurelia_sp.	GTGTGCTCTTAATTGAGTGTGCGTAGGATTGCGACGTTTACTTTGAAAAAATTAGAGTG	779

Chrysaora_lactea	TTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTT	814
Chrysaora_sp	TTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTT	834
Chrysaora_melanaster	TTCAAAGCAGGCTAACCCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTT	814
Chrysaora_fuscescens	TTCAAAGCAGGCTAACCCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTT	816
Cyanea_capillata	TTCAAAGCAGGCTGACCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTT	818
Aurelia_sp.	TTCAAAGCAGGCTAACCCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTT	839

Chrysaora_lactea	CTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATTTCGT	874
Chrysaora_sp	CTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATTTCGT	894
Chrysaora_melanaster	CTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATTTCGT	874
Chrysaora_fuscescens	CTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATTTCGT	876
Cyanea_capillata	CTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATTTCGT	878
Aurelia_sp.	CTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATTTCGT	899

Chrysaora_lactea	ATTTCGTTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGCATT	934
Chrysaora_sp	ATTTCGTTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGCATT	954
Chrysaora_melanaster	ATTTCGTTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGCATT	934
Chrysaora_fuscescens	ATTTCGTTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGCATT	936
Cyanea_capillata	ATTTCGTTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGCATT	938
Aurelia_sp.	ATTTCGTTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGCATT	959

Chrysaora_lactea	TGCCAAGAAATGTTTTTCAATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATAC	994
Chrysaora_sp	TGCCAAGAAATGTTTTTCAATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATAC	1014
Chrysaora_melanaster	TGCCAAGAAATGTTTTTCAATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATAC	994
Chrysaora_fuscescens	TGCCAAGAAATGTTTTTCAATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATAC	996
Cyanea_capillata	TGCCAAGAAATGTTTTTCAATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATAC	998
Aurelia_sp.	TGCCAAGAAATGTTTTTCAATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATAC	1019

Chrysaora_lactea	CGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACC	1054
Chrysaora_sp	CGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACC	1074
Chrysaora_melanaster	CGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACC	1054
Chrysaora_fuscescens	CGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACC	1056
Cyanea_capillata	CGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACC	1058
Aurelia_sp.	CGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACC	1079

Chrysaora_lactea	CCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAG	1114
Chrysaora_sp	CCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAG	1134
Chrysaora_melanaster	CCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAG	1114
Chrysaora_fuscescens	CCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAG	1116
Cyanea_capillata	CCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAG	1118
Aurelia_sp.	CCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAG	1139

Chrysaora_lactea	CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGAGCCTGCGGCTTAATTT	1174
Chrysaora_sp	CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGAGCCTGCGGCTTAATTT	1194
Chrysaora_melanaster	CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGAGCCTGCGGCTTAATTT	1174
Chrysaora_fuscescens	CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGAGCCTGCGGCTTAATTT	1176
Cyanea_capillata	CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGAGCCTGCGGCTTAATTT	1178
Aurelia_sp.	CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGAGCCTGCGGCTTAATTT	1199

Chrysaora_lactea	GACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCT	1234
Chrysaora_sp	GACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCT	1254
Chrysaora_melanaster	GACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCT	1234
Chrysaora_fuscescens	GACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCT	1236
Cyanea_capillata	GACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCT	1238
Aurelia_sp.	GACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCT	1259

Chrysaora_lactea	CTTTCTTGATTCTATGGTGGTGTGTCATGGCCGTTCTTAGTTGGTGGAGTGATTGTCT	1294
Chrysaora_sp	CTTTCTTGATTCTATGGTGGTGTGTCATGGCCGTTCTTAGTTGGTGGAGTGATTGTCT	1314
Chrysaora_melanaster	CTTTCTTGATTCTATGGTGGTGTGTCATGGCCGTTCTTAGTTGGTGGAGTGATTGTCT	1294
Chrysaora_fuscescens	CTTTCTTGATTCTATGGTGGTGTGTCATGGCCGTTCTTAGTTGGTGGAGTGATTGTCT	1296
Cyanea_capillata	CTTTCTTGATTCTATGGTGGTGTGTCATGGCCGTTCTTAGTTGGTGGAGTGATTGTCT	1298
Aurelia_sp.	CTTTCTTGATTCTATGGTGGTGTGTCATGGCCGTTCTTAGTTGGTGGAGTGATTGTCT	1319

Chrysaora_lactea	GGTTAATTCCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTC	1354
Chrysaora_sp	GGTTAATTCCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTC	1374
Chrysaora_melanaster	GGTTAATTCCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTC	1354
Chrysaora_fuscescens	GGTTAATTCCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTC	1356
Cyanea_capillata	GGTTAATTCCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTC	1358
Aurelia_sp.	GGTTAATTCCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTC	1379
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Chrysaora_lactea	GTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGT	1414
Chrysaora_sp	GTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGT	1434
Chrysaora_melanaster	GTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGT	1414
Chrysaora_fuscescens	GTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGT	1416
Cyanea_capillata	GTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGT	1418
Aurelia_sp.	GTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGT	1439

Chrysaora_lactea	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAGCGAGTC	1474
Chrysaora_sp	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAGCGAGTC	1494
Chrysaora_melanaster	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAGCGAGTC	1474
Chrysaora_fuscescens	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAGCGAGTC	1476
Cyanea_capillata	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAGCGAGTC	1478
Aurelia_sp.	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAGCGAGTC	1499
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Chrysaora_lactea	TTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGATAGATC	1534
Chrysaora_sp	TTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGATAGATC	1554
Chrysaora_melanaster	TTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGATAGATC	1534
Chrysaora_fuscescens	TTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGATAGATC	1536
Cyanea_capillata	TTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGATAGATC	1538
Aurelia_sp.	TTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGATAGATC	1559

Chrysaora_lactea	ATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT	1594
Chrysaora_sp	ATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT	1614
Chrysaora_melanaster	ATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT	1594
Chrysaora_fuscescens	ATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT	1596
Cyanea_capillata	ATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT	1598
Aurelia_sp.	ATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGCGTT	1619

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Chrysaora_lactea      GATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGC 1654
Chrysaora_sp          GATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGC 1674
Chrysaora_melanaster GATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGC 1654
Chrysaora_fuscescens GATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGC 1656
Cyanea_capillata     GATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGC 1658
Aurelia_sp.          GATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGC 1679
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Chrysaora_lactea      GAGACCTTCGGATTGGCACCGCTTTGGCCGGCAACGGCCGAGGTGGATCGCCGAGAAGTC 1714
Chrysaora_sp          GAGACCTTCGGATTGGCACCGCTTCGGCCGGCAACGGCTGAGGTGGAAACGCCGAGAAGTC 1734
Chrysaora_melanaster GAGACCTTCGGATTGGCGCCCTTTTCGGCCGGCAACGGCCGAGTGGATAGCCGAGAAGTC 1714
Chrysaora_fuscescens GAGACCTTCGGATTGGCGCCCGCTCAGCCGGCAACGGCCGAGCGGATAGCCGAGAAGTC 1716
Cyanea_capillata     GAGACCTTCGGATTGGCACTGCCTTAGCCGGAAACGGCTGAAGTGGATAGCCGAGAAGTC 1718
Aurelia_sp.          GAGACCTTCGGATTGGCGCCGCTTTTCGGCCCGCAAGGGCTGAAGTGGATAGCCGAGAAGTC 1739
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Chrysaora_lactea      GTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTC----- 1762
Chrysaora_sp          GTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCGTAGGTGAACCT 1794
Chrysaora_melanaster GTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTC----- 1762
Chrysaora_fuscescens GTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTC----- 1764
Cyanea_capillata     GGTCTAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTC----- 1766
Aurelia_sp.          GCTCTAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCGTAGGTGAACCT 1799
*

Chrysaora_lactea      -----
Chrysaora_sp          GCA----- 1797
Chrysaora_melanaster -----
Chrysaora_fuscescens -----
Cyanea_capillata     -----
Aurelia_sp.          GCGGAAGGATCATTA 1814

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Figure 6: ClustalW2 alignment 1. Alignment of 6 cnidarian species to design primer sets 1-3 for 18S rDNA amplification. Regions in colored boxes indicate primers used in primer sets 1-3. Red box defines primer 3 (F), blue box defines primer 1 (internal F), green box defines primer 2 (internal R), and purple boxes define primer 4 (R).

Primer Set	Forward (5' → 3')	Reverse (5' → 3')
1	Primer 1 CTCGTAGTTGGATTTTCGGGA	Primer 2 AACTAAGAACGGCCATGCAC
2	Primer 3 AACCTGGTTGATCCTGCCAGT	Primer 2 AACTAAGAACGGCCATGCAC
3	Primer 1 CTCGTAGTTGGATTTTCGGGA	Primer 4 GATCCTTCTGCAGGTTCACCTAC

Table 3: Summary of Primer Sets 1-3. Colors are consistent with Figure 5 to identify primer positions.

Set 4 primers (5 and 8) were used to amplify the 5' end of the 28S rDNA sequence and should yield a product of 1138 bp, approximately one-third of the 28S rDNA sequence. Relative positions of primers were identified through a Clustal Alignment of known cnidarian 28S rDNA sequences (Fig. 7).

Set 4 primers are:

Primer 5 (Forward) ACCCGCTGAATTTAAGCATA

Primer 8 (Reverse) GAAACTTCGGAGGGAACCAGCTAC

```
Aurelia_sp      TTTGACCTCAGATCAGGCAAGACTACCCGCTGAATTTAAGCATAATTAATAAGCGGAGGAA 60
Cyanea_capillata -----
Chrysaora_fuscescens -----
Chrysaora_melanaster -----
Chrysaora_lactea -----
Chrysaora_sp    -----C 1

Aurelia_sp      AAGAAACTAACAAGGATTCCCTCAGTAACGGCGAGTGAAGTGGGAATGGCTCAAGCTTGA 120
Cyanea_capillata -----
Chrysaora_fuscescens -----TTCCCTCAGTAACGGCGAGTGAAGTGGGAACAGCTCAAGCTTGA 44
Chrysaora_melanaster -----
Chrysaora_lactea -----
Chrysaora_sp    GAGAAAGAACTACAAGGATCCTCAGTAACGGCGAGTGAAGTGGGAACAGCTCAAGCTTGA 61

Aurelia_sp      AATCTCTGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTTCCCGCGGATCTA 180
Cyanea_capillata -----CTGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTTCCCGCGGACCTT 55
Chrysaora_fuscescens AATCTCTGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTTCCCGCGGACCTT 104
Chrysaora_melanaster -----TGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTTCCCGGTGGACCTT 54
Chrysaora_lactea    -----CTGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTCCCGCGGACCTT 55
Chrysaora_sp    AATCTCTGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTCCCGGTGGACCTT 121
                *****

Aurelia_sp      TCCTGCCTAAGTTGCTTGGAACAGCACATCAGAGAGGGTGACAATCCCGTTTGTGGCAGG 240
Cyanea_capillata -----CCCTGCCTAAGTTGCTTGGAACAGCACATCAGAGAGGGTGACAATCCCGTTTGTGGCAGG 115
Chrysaora_fuscescens -----TCCTGTCTAAGTTGCTTGGAACAGCACATCAGAGAGGGTGACAATCCCGTTTGTGGCAGG 164
Chrysaora_melanaster TCCTGTYTRAGTTGCTTGGAACAGSACATCAGAGAGGGTGACAATCCCGTTTGTGGCAGG 114
Chrysaora_lactea    TCCTGTCTAAGTNNCTTGGAACAGCANNTCGGAGAGGGTGACAATCCCGTTTGTGGCAGG 115
Chrysaora_sp    TCCTGTCTAAGTTGCTTGGAACAGCACATCGGAGAGGGTGACAATCCCGTTTGTGGCAGG 181
                **** *

Aurelia_sp      GCAGACCGTTTACGATACGCTTTCCATGAGTCGGGTGCTTGGGAATGCAGCCCAAAT 300
Cyanea_capillata -----GCGGGCCGTTTACGATACGCTTTCCATGAGTCGGGTGCTTGGGAATGCAGCCCAAAT 175
Chrysaora_fuscescens ACAGGCTGCCAACGATACGCTTTCCATGAGTCGGGTGCTTGGGAATGCAGCCCAAAT 224
Chrysaora_melanaster AAAGGCTGCCAACGATACGCTTTCCATGAGTCGGGTGCTTGGGAATGCAGCCCAAAT 174
Chrysaora_lactea    ACTGGCTGCCAACGATACGCTTTCCATGAGTCGGGTGCTTGGGAATGCAGCCCAAAT 175
Chrysaora_sp    ACTGGCTGCCAACGATACGCTTTCCATGAGTCGGGTGCTTGGGAATGCAGCCCAAAT 241
                *** *

Aurelia_sp      GGTGGTAAACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAGTACCGT 360
Cyanea_capillata -----GGTGGTAAACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAGTACCGT 235
Chrysaora_fuscescens GGTGGTAAACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAGTACCGT 284
Chrysaora_melanaster GGTGGTAAACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAGTACCGT 234
Chrysaora_lactea    GGTGGTAAACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAGTACCGT 235
Chrysaora_sp    GGTGGTAAACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAGTACCGT 301
                *****

Aurelia_sp      GAGGGAAGATGAAAAGAACTTTGAAAAGAGAGTTAAAAAGTGCCTGAAACCGTTAGAGG 420
Cyanea_capillata -----GAGGGAAGATGAAAAGAACTTTGAAAAGAGAGTTAAAAAGTGCCTGAAACCGTTAGAGG 295
Chrysaora_fuscescens GAGGGAAGATGAAAAGAACTTTGAAAAGAGAGTTAAAAAGTGCCTGAAACCGTTAGAGG 344
Chrysaora_melanaster GAGGGAAGATGAAAAGAACTTTGAAAAGAGAGTTAAAAAGTGCCTGAAACCGTTAGAGG 294
Chrysaora_lactea    GAGGGAAGATGAAAAGAACTTTGAAAAGAGAGTTAAACAGTGCCTGAAACCGTTAGAGG 295
Chrysaora_sp    GAGGGAAGATGAAAAGAACTTTGAAAAGAGAGTTAAACAGTGCCTGAAACCGTTAGAGG 361
                *****

Aurelia_sp      GGAAGCGGATGCAGTTAGCAATGCTTTGCGAGATTACGGCGGTGCTCGGCCAGACGTG 480
Cyanea_capillata -----GGAACCGGATGCAGTTAGCAATGCTTTCGGCGAGATTACAGCGGCGCTTGGCCAGACGTG 355
Chrysaora_fuscescens GGAACCGGATGCAGTTAGCAATGCTTTCGGTGAGATTACGGCGGTGCTTGGCTGGACGTG 404
Chrysaora_melanaster GGAACCGGATGCAGTTAGCAATGCTTTCGGCGAGATTACGGTGGTTCGTTAGACGTG 354
Chrysaora_lactea    GGAACCGGATGCAGTTAGCAATGCTTTCGGCGAGATTACGGCGATCGTTTCGCTGGACTTG 355
Chrysaora_sp    GGAACCGGATGCAGTTAGCAATGCTTTCGGCGAGATTACGGCGATCGTTTCGCTGGACTTG 421
                **** *

Aurelia_sp      CAGTTGATGGATCCGAATGGACTGTCGCTGTACGGCCGGGTCTTGTGCCCTGCGCACTTC 540
Cyanea_capillata -----CAGTGTATGGATCCGAATGGACTGTCGCTGTACGGCCGGCTTGTGCTCGTCGCACTTT 415
Chrysaora_fuscescens CAGTGTATGGATCCGAATGGACTGTCGCTGTACGGCCGGTCTTGTGTTTCGTCGCACTTC 464
Chrysaora_melanaster CAGTGTATGGATCCGATGGACTGTCGCTGTACGGCCGGCTTGTGTTTCATCGCACTTC 414
Chrysaora_lactea    CAGCGTGGGATCCGAATGGACCGTTCGCTGTTTCGGCTCGGTGTTGTGTTTCGTCGCACTTC 415
Chrysaora_sp    CAGCGTAGGATCCGAATGGACCGTTCGCTGTTTCGGCTCGGTGTTGTGTTTCGTCGCACTTC 481
                **** *
                *****
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Aurelia_sp TCGCTGGAGCGCGTCAACAGCTGCTGGGCTGGTCGACACGGTCAAGTGAAGGTAAGCA 600
 Cyanea_capillata TCGTCGAGGCGCGTCAACAGCTGTTGGGACTGAACGACAAGGCTCCGAGGAAGGTAACCG 475
 Chrysaora_fuscescens TCGTCGGGCGCGTCAACAGCTGCTTTGTTTGTAGTAGGCCACTCGGGGAAGGTAGGCG 524
 Chrysaora_melanaster TCGTTGGGCGCGTCAACAGCTGCTTTGTCTGAGTGATAAGGCGCCAGGAAGGTAGGCG 474
 Chrysaora_lactea TCGTCGGAGCGCGTCAACAGTTGCTCCGTCTGGGCGACAAGGCACCCAGGAAGGTAGGCG 475
 Chrysaora_sp TCGTCGGAGCGCGTCAACAGTTGCTCTGTCTGGGCGAAAAGGCATCCAGGAAGGTAGGCG 541
 *** * ***** * * * * * * * * * *

Aurelia_sp TCCAGCTTG-CTGGCTGTTGTTATATCCACTC--GTACCTAGATCGGGCTTGGCAGAGGC 657
 Cyanea_capillata TGTACTTCG-GTATGCGGCGTTATAGCCTCAGA-GTACCCAGTTCCGTCCTGGCAGAGGC 533
 Chrysaora_fuscescens CGTGTTCG-ATGCGCGTTGTTATAGCCCGTGTGTCGCTAGCTCGGACATGGCAGAGGC 583
 Chrysaora_melanaster TGTATCTTGTATATGTGCTGTTATAGCCTGTGGTGGTCCCAACTCGGACAGGGCAGAGGC 534
 Chrysaora_lactea GGTACTTCG-GTACGTGCTGTTATAGCCTGTGGTGATACAAGCTCGGACGGGCGAGAGGC 534
 Chrysaora_sp GGTACTTCG-GTACGTGCTGTTATAGCCTGTGGTGATACAAGCTCGGACGGGCGAGAGGC 600
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Aurelia_sp GAGCGGTGCATGCTCTCTCGAGAGGGCTGGCGTCCTGTTCCGTTTAC--GTAGCCGGC 715
 Cyanea_capillata GAGCGGTGCATGCCCTC-----GGGCTGGCGTACC-TCTCTTGGCTGGGTGTCGAC 584
 Chrysaora_fuscescens GAACGGTGCATGCTCTTCTTCGGAGGGGCTGGCGTCCT--TCTCTGACTGTGTCGAC 641
 Chrysaora_melanaster GAGCGGTGCATGCTCTTCTTCGGAGGGGCTGGCGCCCT--TCTCTTGGCCGGATGTCGAC 592
 Chrysaora_lactea GAACGGTGCATGCTCTTCTTCGGAGAGGGCTGGCACCCCT--CTTCTTGACAGGATGTCGAC 592
 Chrysaora_sp GAACGGTGCATGCTCTTCTTCGGAGAGGGCTGGCGCCCT--CTTCTTGACAGGATGTCGAC 658
 * * * * * * * * * *

Aurelia_sp TTGGATGCACTGCGTGCAGTACGT-----TCTTGCCGGTGAAAC-GGCGTGCAGTCAAC 768
 Cyanea_capillata CATGACGGACTGCTTGCAGTGCCTGAACTGCTCCTGGTCATTAGGGGTGTAATC--- 641
 Chrysaora_fuscescens CATGGCGGACTGCTTGCAGTGCCTGAACTGCTTTGGGTGCATACAGGAG---GTCAAC 697
 Chrysaora_melanaster CATGGCGGACTGCTTGCAGTGCCTGAACTGCTTTGGGTGATTCGGGAG---GTCTAC 648
 Chrysaora_lactea CATGGCGGACTGCTTGCAGTGCCTGAAATGCTTTTGTCACTCGGGAG---GATCGT 648
 Chrysaora_sp CATGGCGGACTGCTGTCAGTGCCTGAAATGCTTTTGTCACTCGGGAG---GATCGT 714
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Aurelia_sp GCACTCGATGCACCATGGTTGTTGGCGGCCATATGGCTTTATCCGACCCGCTCTTGAAACA 828
 Cyanea_capillata GCACACTATGCACCTTGGTTGTTGGCGGCCATATGGCTTTATCCGACCCGCTCTTGAAACA 701
 Chrysaora_fuscescens GCACACTATGCACCTAGGTTGTTGGCGGCCATATGGCTTTCATCTGACCCGCTCTTGAAACA 757
 Chrysaora_melanaster GCACACTATGCACCTGTTGTTGGCGGCCATATGGCTTTCATCTGACCCGCTCTTGAAACA 708
 Chrysaora_lactea GCACAC-ATGCACCTTGGTTGTTGGCGGCCATATGGCTTTCATCCGACCCGCTCTTGAAACA 707
 Chrysaora_sp GCACAC-ATGCACCTTGGTTGTTGGCGGCCATATGGCTTTCATCCGACCCGCTCTTGAAACA 773
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Aurelia_sp C-GGACCAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGACCGAAACCCAGAGCGCAA 887
 Cyanea_capillata C-GGACCAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGACCGAAACCCAGGACCAA 760
 Chrysaora_fuscescens C-GGACCAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGACTGAAACCCAAAGGCACAA 816
 Chrysaora_melanaster C-GGACCAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGACTGAAACCCAAAGGCACAA 767
 Chrysaora_lactea C-GGACCAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGATCGAAACCCAGAGGCACAA 766
 Chrysaora_sp CCGGACCAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGATCGAAACCCAAAGGCACAA 833
 * * * * * * * * * *

Aurelia_sp TGAAAGTGAAGGCTTCTTGTGAGGCTGAGGTGAGATCTTGTCTCTACGCGAGTGAGGCG 947
 Cyanea_capillata TGAAAGTGAAGGCTGCCTAGCA--GCTGAGGTGAGATCTTCTGTTCTCCGTGGATGAGGCG 818
 Chrysaora_fuscescens TGAAAGTGAAGGCTGCCTAGCA--GCTGAGGTGAGATCTTCTGTTCTACGTGATCGAGGCG 874
 Chrysaora_melanaster TGAAAGTGAAGGCTGCCTAGCA--GCTGAGGTGAGATCTTCTGTTCTGCGTGATCGAGGCG 825
 Chrysaora_lactea TGAAAGTGAAGGCTGCCTAGCA--GCCGAGGTGAGATCTTCTGTTCTGCGGTTTGAAGCG 824
 Chrysaora_sp TGAAAGTGAAG-CTGCCTAGCA--GCCGAGGTGAGATCTTCTGTTCTGCGGTTTGAAGCG 890
 * * * * * * * * * *

Aurelia_sp CATCATCGACCGACCTATTCTACTCTTAGGAAGGTTTGAAGAGCGTACATGTTGGGA 1007
 Cyanea_capillata CATCATCGACCGACCTATTCTACTCTTAGGAAGGTTTGAAGAGCGCAATGTTGGGA 878
 Chrysaora_fuscescens CATCATCGACCGACCTGTTCTACTCTTAGGAAGGTTTGAAGAGCGCATCTGTTGGGA 934
 Chrysaora_melanaster CATCATCGACCGACCTATTCTACTCTTAGGAAGGTTTGAAGAGCGCATCTGTTGGGA 885
 Chrysaora_lactea CATCATCGACCGACCTATTCTACTCTTAGGAAGGTTTGAAGAGCGCATCTGTTGGGA 884
 Chrysaora_sp CATCATCGACCGACCTATTCTACTCTTAGGAAGGTTTGAAGAGCGCATCTGTTGGGA 950
 * * * * * * * * * *

Aurelia_sp CCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGC 1067
 Cyanea_capillata CCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGC 938
 Chrysaora_fuscescens CCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGC 994
 Chrysaora_melanaster CCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGC 945
 Chrysaora_lactea CCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGC 944
 Chrysaora_sp CCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGC 1010
 * * * * * * * * * *

Aurelia_sp TCGTAGCGATTCTGACGTGCAAAATCGATCGTCGAACCTGGGTATAGGGGCGAAAGACTAA 1127
 Cyanea_capillata TCGTAGCGATTCTGACGTGCAAAATCGATCGTCGAACCTGGGTATAGGGGCGAAAGACTAA 998
 Chrysaora_fuscescens TCGTAGCGATTCTGACGTGCAAAATCGATCGTCGAACCTGGGTATAGGGGCGAAAGACTAA 1054
 Chrysaora_melanaster TCGTAGCGATTCTGACGTGCAAAATCGATCGTCGAACCTGGGTATAGGGGCGAAAGACTAA 1005
 Chrysaora_lactea TCGTAGCGATTCTGACGTGCAAAATCGATCGTCGAACCTGGGTATAGGGGCGAAAGACTAA 1004
 Chrysaora_sp TCGTAGCGATTCTGACGTGCAAAATCGATCGTCGAACCTGGGTATAGGGGCGAAAGACTAA 1070
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Aurelia_sp          TCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCTCAGGATAGCAGGAACTCG--G 1185
Cyanea_capillata    TCGAACCATCTA----- 1010
Chrysaora_fuscescens TCGAACCATCTA----- 1066
Chrysaora_melanaster TCGAACCATCTA----- 1017
Chrysaora_lactea     TCGAACCATCTA----- 1016
Chrysaora_sp        TCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCTCAGGATAGCAGGGACTCGACG 1130
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Figure 7: ClustalW2 Alignment 2. Alignment of 6 cnidarian species to design primer set 4 for 28S rDNA amplification. Regions in black boxes indicate primers used in primer set 5.

Primer sets 5 and 6 were designed based on sequencing the products of the first four sets (Consensus sequences 2-4, see Results section).

Sample Collection and DNA Extraction

Genomic DNA was isolated from frozen *Chrysaora quinquecirrha* (both adult medusa and polyps) using the jellyfish CTAB/NaCl protocol (Appendix, page 106). This study focused on the use of DNA samples from Barnegat Bay. DNA samples from Navesink River and Chesapeake Bay were also used.

PCR Amplification

Sequences were amplified using ChoiceTaq Mastermix (Denville Scientific) with the primer sets defined above in an ABI Veriti Thermal Cycler. PCR reactions consisted of: 10 µl (1X) ChoiceTaq. Mastermix, 1 µl (0.5µM) of each primer, and 1 µl of DNA in a total 20 µl reaction.

PCR conditions varied for each primer set in terms of the annealing temperature and extension time, which was due to primer melting temperatures and fragment size amplified, respectively. PCR conditions for each reaction as follows:

Reaction #1 PCR conditions were: initial denaturation at 95°C for 1 min (one cycle); denaturation at 95°C for 15 sec, annealing at 55°C for 20 sec, extension at 72°C for 40 sec (35 cycles); final extension at 72°C for 7 min (one cycle); soak at 4°C until removed.

Reaction #2 PCR conditions were: initial denaturation at 95°C for 1 min (one cycle); denaturation at 95°C for 15 sec, annealing at 55°C for 20 sec, extension at 72°C for 80 sec (35 cycles); final extension at 72°C for 7 min (one cycle); soak at 4°C until removed.

Reaction #3 PCR conditions were: initial denaturation at 95°C for 1 min (one cycle); denaturation at 95°C for 15 sec, annealing at 55°C for 20 sec, extension at 72°C for 70 sec (35 cycles); final extension at 72°C for 7 min (one cycle); soak at 4°C until removed.

Reaction #4 PCR conditions were: initial denaturation at 95°C for 1 min (one cycle); denaturation at 95°C for 15 sec, annealing at 54°C for 20 sec, extension at 72°C for 70 sec (35 cycles); final extension at 72°C for 7 min (one cycle); soak at 4°C until removed.

Successful amplification was confirmed by gel electrophoresis using a 1% agarose gel (in TAE) stained with SYBR Safe DNA gel stain. Gels were run for

approximately 40 minutes at 100V. Hi/lo DNA marker from Minnesota Molecular (<http://mnmolecular.com/>) was used to indicate band size.

Sequencing and Analysis

Samples with successful amplification were sequenced on an ABI 3130 Genetic Analyzer. One μ l of each PCR reaction was sequenced using either the forward or reverse primer with the BigDye Terminator Ready Reaction Kit (Version 3.1) from Applied Biosystems. Reactions were mixed and cleaned prior to loading onto sequencer (Appendix, page 108). Reactions were performed according to manufacturer's instructions (Applied Biosystems Ready Reaction Kit).

Resulting electropherograms were viewed and edited for accuracy with CodonCode Aligner (ver. 4.1.1; <http://codoncode.com/>) and BLASTn searches. Ends were clipped and overlapping sequences were assembled together to create consensus sequences. Sequences were compared to the GENBANK database by performing a BLASTn search to confirm the correct region had been sequenced. Comparisons between species were visualized through ClustalW2 alignments.

Results

Original Primers and Sequencing

Relative positions of previously designed primers are predicted in Figure 8, where each primer was aligned with 45S rDNA cassette of *Aurelia* sp. (EU276014.1).

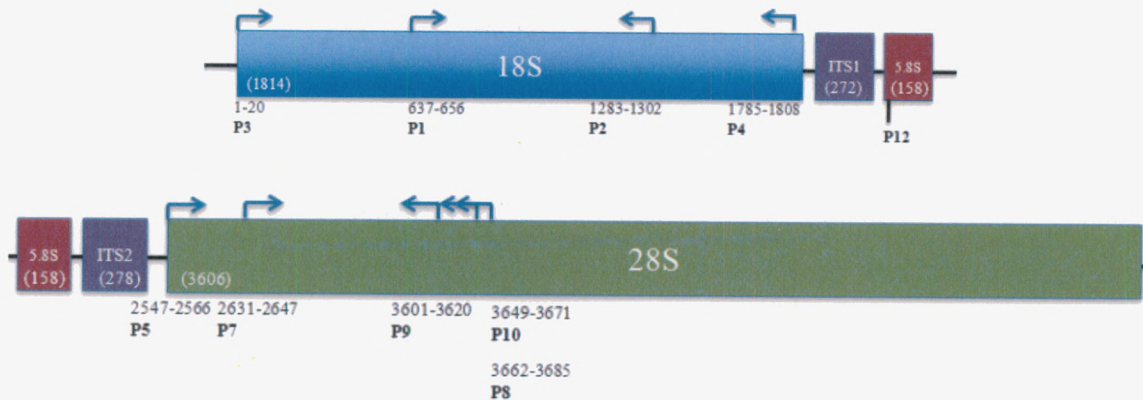


Figure 8: Relative Positions of Previously Designed Primers on Linear Maps of 18S (top) and 28S (bottom) ribosomal DNA in *Aurelia* sp. (EU276014.1). Numbers in parentheses indicate nucleotide size of 18S, ITS1, 5.8S, ITS2, and 28S in *Aurelia*. Arrows indicate direction of primer (forward or reverse).

Set 1 primers (1 and 2) were used to amplify an internal region of 18S rDNA of approximately 665 bp in length. Successful amplification was confirmed on 1% agarose gels. A distinct single band of approximately 650 bp is present in all samples from Barnegat Bay: Lanes 2-3, 6-9, 11-18, and 20-21 (Fig. 9). Of the Chesapeake Bay samples, no bands are present in lane 4, and three bands are seen in lane 5 of approximately 650 bp, 350 bp, and 150 bp. Lanes 2, 5, 6, 11, 16, and 20 were used for sequencing to determine 18S sequences.

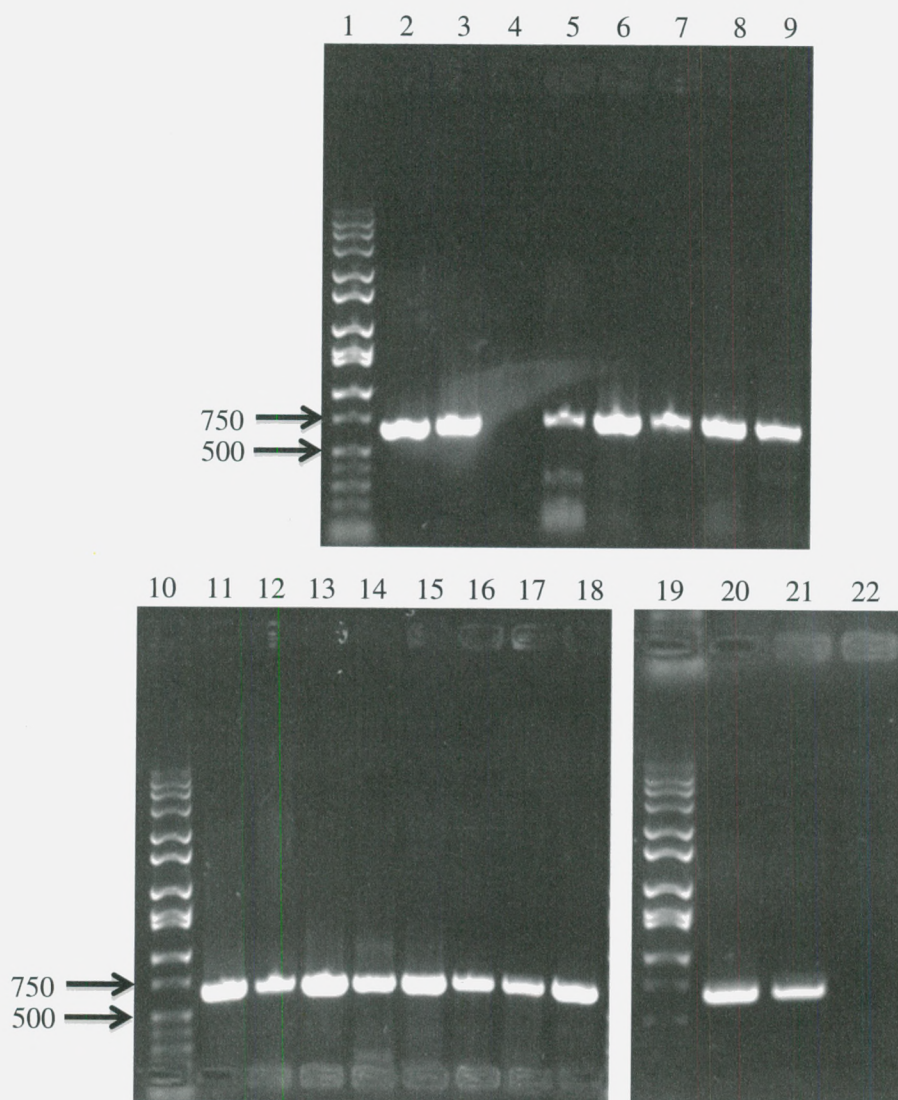


Figure 9: Agarose gels of Primer Set 1. Lanes 1, 10, 19 = Hi/Lo marker; Lanes 2-3, 6-9, 11-18, 20-21 = Barnegat Bay *CQ*; Lanes 4-5 = Chesapeake Bay *CQ*; Lane 22 = sterile deionized water (negative control). Lanes 2, 5, 6, 11, 16, and 20 were used for sequencing.

Set 2 primers (2 and 3) generated a distinct band of approximately 1350 bp in all Barnegat Bay samples: lanes 2-4, 7-9, 11-18, and 20-22 (Fig. 10). This band was not observed in Chesapeake Bay samples: lanes 5-6. Instead a faint band of approximately 150 bp was observed in these lanes. Smaller faint bands are also seen in lanes 4, 7-9, 11-16, 18, and 20-22. Due to the intensity of the 1350 bp fragment as compared to smaller bands, lanes 2, 7, 12, 16, and 21 were used for sequencing to determine 18S sequences.

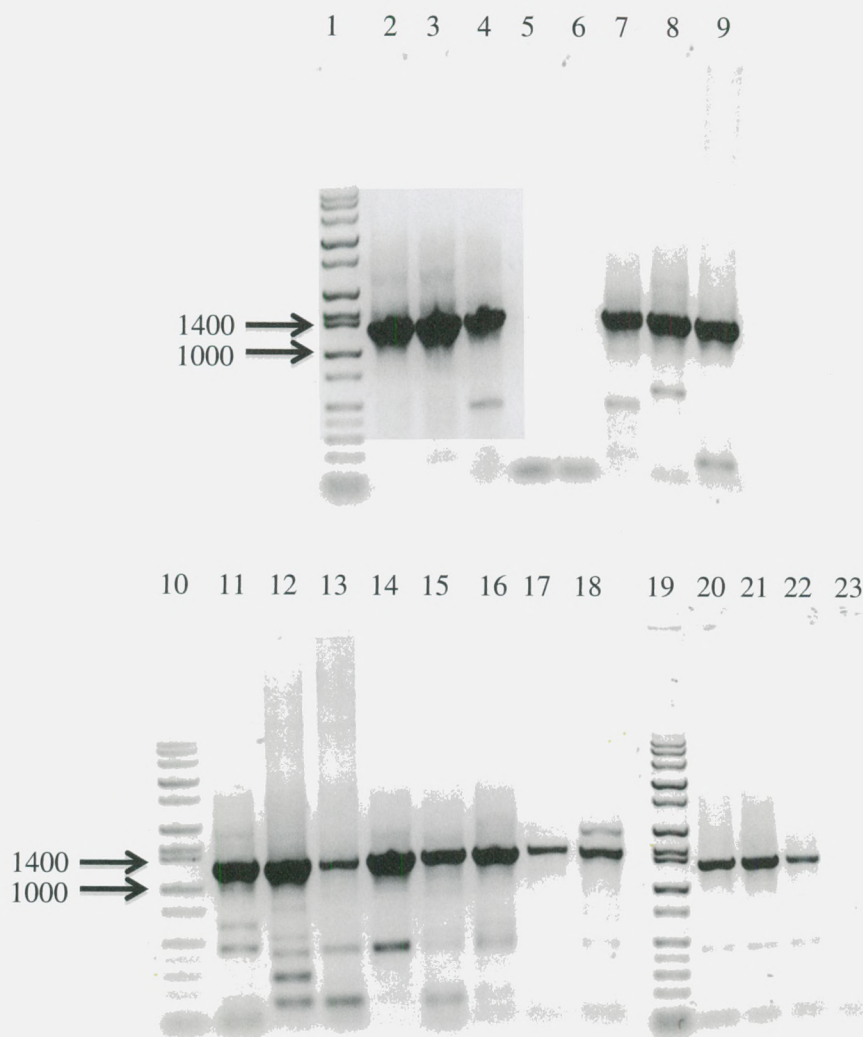


Figure 10: Agarose gels of Primer Set 2. Lanes 1, 10, 19 = Hi/lo marker; Lanes 2-4, 7-9, 11-18, 20-22 = Barnegat Bay *CQ*; Lanes 5-6 = Chesapeake Bay *CQ*; Lane 23 = sterile deionized water (negative control). Lanes 2, 7, 12, 16, and 21 were used for sequencing.

Set 3 primers (1 and 4) generated a distinct band of approximately 1200 bp in all Barnegat Bay samples: lanes 2-4, 7-9, 11-18, and 20-22 (Fig. 11). This band was not observed in Chesapeake Bay samples: lanes 5-6. Instead a faint band of approximately 150 bp was observed in these lanes. Smaller faint bands are also seen in lanes 4, 7-9, 11-12, 14, and 20-22. Due to the intensity of the 1200 bp fragment, lanes 2, 7, 12, 16, and 21 were used for sequencing to determine 18S sequences.

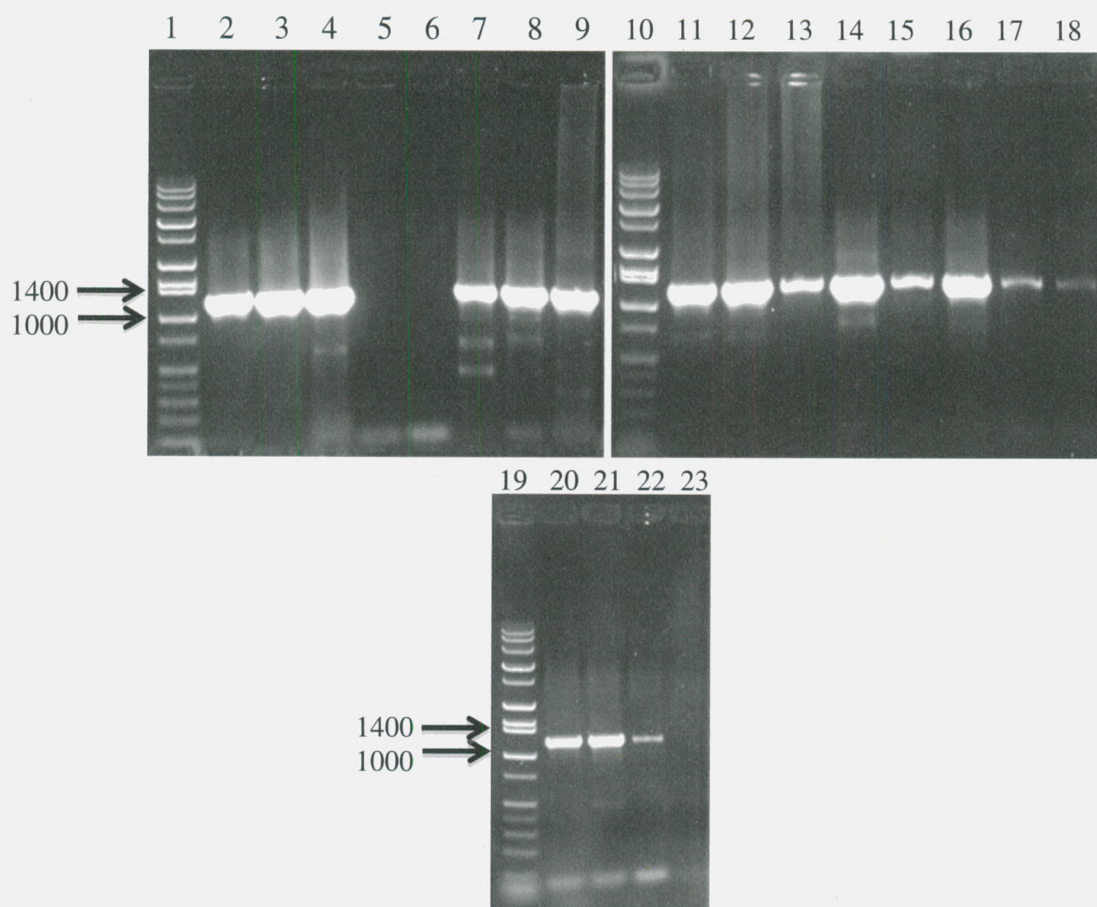


Figure 11: Agarose gels of Primer Set 3. Lanes 1, 10, 19 = Hi/lo marker; Lanes 2-4, 7-9, 11-18, 20-22 = Barnegat Bay *CQ*; Lanes 5-6 = Chesapeake Bay *CQ*; Lane 23 = sterile deionized water (negative control). Lanes 2, 7, 12, 16, and 21 were used for sequencing.

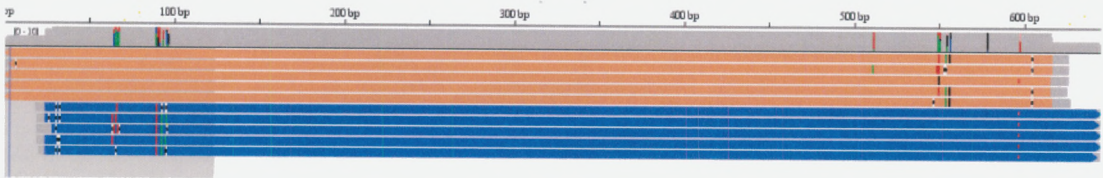
Sequencing reactions for primer sets 1-3 were run and viewed as described above. The number of nucleotides (nts) acquired from each reaction from Barnegat Bay samples is listed in Table 4. There were regions of large peaks within the first 100 nucleotides of each electropherogram. Regions with large peaks were identified by viewing the opposite primer sequence data. From set 1, two consensus sequences were generated: one from Barnegat Bay samples and one from Chesapeake Bay. For Barnegat Bay, the forward and reverse primer sequences overlapped by 593 bp. 644 bp were generated from set 1 Barnegat Bay samples to create consensus sequence 1 (Fig. 12). Chesapeake

Bay forward and reverse sequence reactions overlapped by 352 bp and were assembled to create consensus sequence 2, a sequence of 640 bp total (Fig. 13).

Samples	Set 1	Set 2	Set 3
1	F=650 nts, R=641 nts	F=896nts, R=871nts	F=882nts, R=882nts
2	F=646 nts, R=641 nts	F=918nts, R=897nts	F=903nts, R=889nts
3	F=654 nts, R=640 nts	F=792nts, R=899nts	F=874nts, R=896nts
4	F=647 nts, R=639 nts	F=819nts, R=914nts	F=864nts, R=905nts
5	F=651 nts, R=642 nts	F=835nts, R=908nts	F=865nts, R=862nts
	F + R Overlap=593 Total Length=644	F + R Overlap=365 Total Length=1229	F + R Overlap=585 Total Length=1093

Table 4: Nucleotides (nts) generated from forward (F) and reverse (R) sequence reactions.

A.



B.

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1-50   GGATTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGT
51-100 CTGTCCTTCTTCGCAAAGACTGCGTGTGCTCTTAATTGAGTGTGCGTAGG
101-150 ATTTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCTGTCC
151-200 GCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTTCTATTTT
201-250 GTTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATT
251-300 CGTATTTTCGTTGTCAGAGGTGAAATTCCTGGATTTACGAAAGACGAACAA
301-350 CTGCGAAAGCATTGCGCAAGAATGTTTTTCATTAATCAAGAACGAAAGTTA
351-400 GAGGCTCGAAGACGATCAGATACCGTCCTAGTTCTAACCATAAACGATGC
401-450 CGACTAGGGATCAGCGGGCGTTATTTTATGACCCCGTTGGCACCTTATGG
451-500 GAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAAC
501-550 TTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA
551-600 TTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATGAC
601-644 AGATTGAGAGCTCTTCTTGATTCTATGGGTGGTGGTGCATGGC

```

Figure 12: Development of consensus sequence 1. A. Alignment of primer set 1 Barnegat Bay sequences using Codon Code Aligner. Orange depicts reverse sequences; Blue depicts forward sequences. Difference in color within each sample depicts variation in nucleotides among sequences, these regions demonstrate large peaks on electropherograms. Shows the overlap of the sequences to produce consensus sequence 1, shown in B.

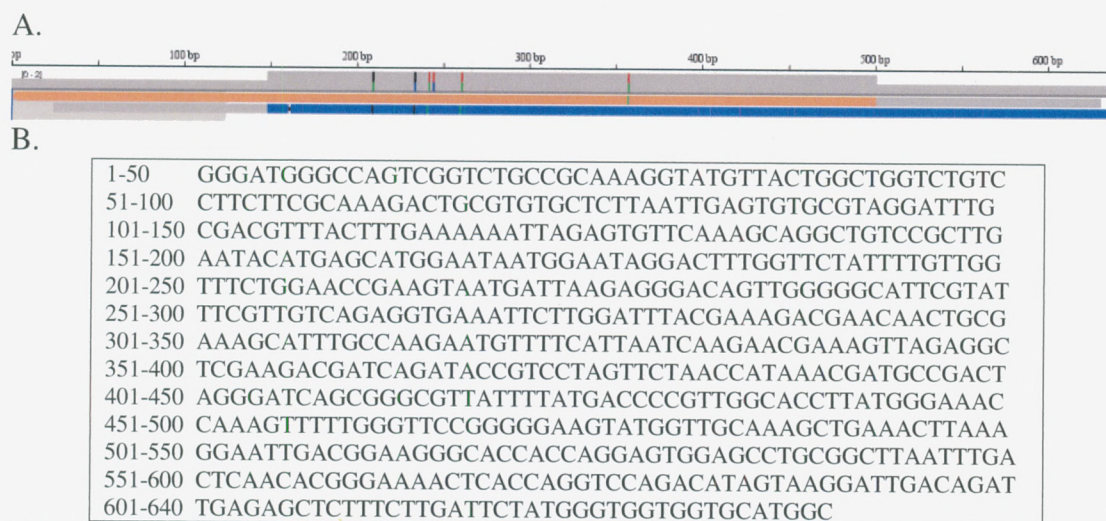


Figure 13: Development of consensus sequence 2. A. Alignment of primer set 1 Chesapeake Bay sequences using Codon Code Aligner. Orange depicts reverse sequence; Blue depicts forward sequence. Difference in color within each sample depicts variation in nucleotides among sequences, these regions demonstrate large peaks on electropherograms. Shows the overlap of the sequences to produce consensus sequence 2, shown in B.

Consensus 1 was run with the BLASTn algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to search for nucleotide matches against any organism. Consensus 1 had a score of ≥ 200 with a 99% maximum identity (Fig. 14). A BLASTn match with *Aurelia* sp. (EU276014.1) confirms that consensus 1 contains an internal fragment of 18S rDNA as it matches with *Aurelia* sp. from position 650-1291 (Fig. 15). 636 nucleotides match in homology with *Aurelia*, and 6 nts varied. When a BLASTn search of consensus 1 against consensus 2 was performed, results indicated high identity, a 99% match (Fig. 16).

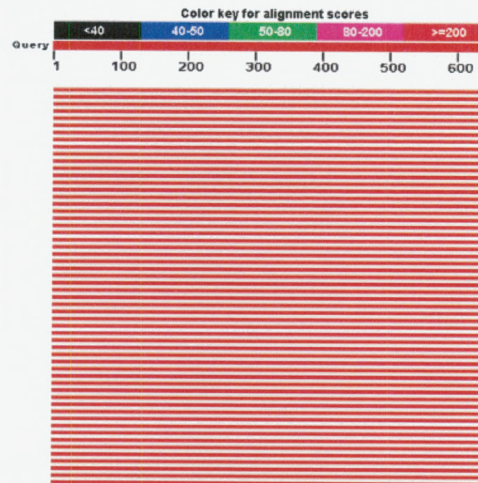
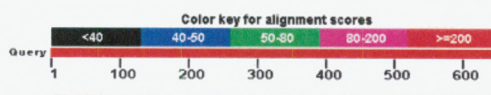


Figure 14: BLASTn matches to consensus sequence 1. The highest match is with *Chrysaora* sp. (AY920769.1) of 99% maximum identity.

Score	Expect	Identities	Gaps	Strand
1128 bits(1250)	0.0	636/642(99%)	1/642(0%)	Plus/Plus
Query 4	TTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTTCTTCG	63		
Sbjct 650	TTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTTCTTCG	709		
Query 64	CAAAGACTGCGTGTGCTCTTAAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAA	123		
Sbjct 710	CAAAGACTGCGTGTGCTCTTAACTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAA	769		
Query 124	AATTAGAGTGTTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAG	183		
Sbjct 770	AATTAGAGTGTTCAAAGCAGGCTAACCCTTGAATACATGAGCATGGAATAATGGAATAG	829		
Query 184	GACTTTGGTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAAGAGGGACAGTTGG	243		
Sbjct 830	GACTTTGGTTCTATTTTGTGGTTTCTAGAACTGAAAGTAATGATTAAAGAGGGACAGTTGG	889		
Query 244	GGGCATTTCGTATTTTCGTTGTCAGAGGTGAAATTCCTGGATTACGAAAGACGAACAACTG	303		
Sbjct 890	GGGCATTTCGTATTTTCGTTGTCAGAGGTGAAATTCCTGGATTACGAAAGACGAACAACTG	949		
Query 304	CGAAAGCATTTGCCAAGAAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGAC	363		
Sbjct 950	CGAAAGCATTTGCCAAGAAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGAC	1009		
Query 364	GATCAGATACCGTCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTA	423		
Sbjct 1010	GATCAGATACCGTCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTA	1069		
Query 424	TTTTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTAT	483		
Sbjct 1070	TTTTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTAT	1129		
Query 484	GGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACAGGAGTGGAGCCTGC	543		
Sbjct 1130	GGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACAGGAGTGGAGCCTGC	1189		
Query 544	GGCTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGA-TGACAG	602		
Sbjct 1190	GGCTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAG	1249		
Query 603	ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGC	644		
Sbjct 1250	ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGC	1291		

Figure 15: BLASTn results of consensus 1 against *Aurelia* sp. This figure shows high identity with *Aurelia* sp. (EU276014.1) from position 650 – 1291.

A.



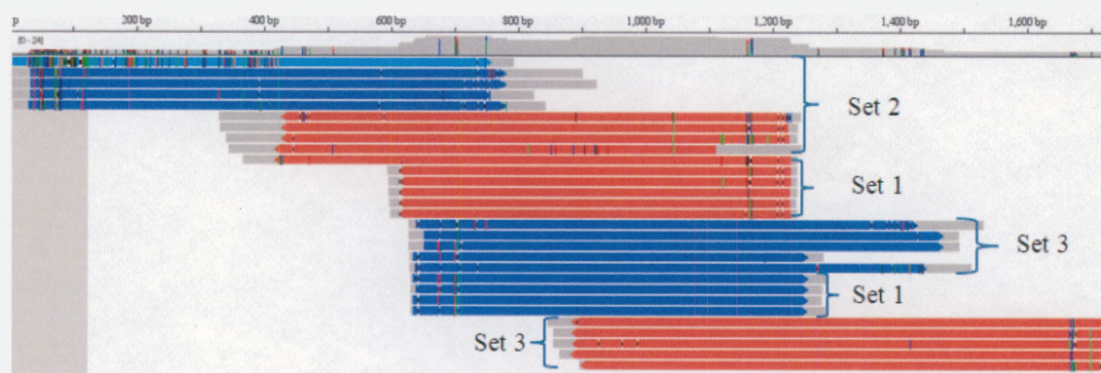
B.

Score	Expect	Identities	Gaps	Strand
1139 bits(1262)	0.0	638/640(99%)	2/640(0%)	Plus/Plus
Query 7	GGGATGGGCCAGTCGGCTGCGCGCAA-GGTATGTTACTGGCTGGTCTGTCTTCTTCGCA	65		
Sbjct 1	GGGATGGGCCAGTCGGCTGCGCGCAAAGGTATGTTACTGGCTGGTCTGTCTTCTTCGCA	60		
Query 66	AAGACTGCGGTGCTCTTAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAAAA	125		
Sbjct 61	AAGACTGCGGTGCTCTTAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAAAA	120		
Query 126	TTAGAGTGTTCAAAGCAGGCTGTCGCTTGAATACATGAGCATGGAATAATGGAATAGGA	185		
Sbjct 121	TTAGAGTGTTCAAAGCAGGCTGTCGCTTGAATACATGAGCATGGAATAATGGAATAGGA	180		
Query 186	CTTTGGTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGG	245		
Sbjct 181	CTTTGGTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGG	240		
Query 246	GCATTCGTATTTCTGTCAGAGGTGAAATCTTGGATTTACGAAAGACGAACAACCTGCG	305		
Sbjct 241	GCATTCGTATTTCTGTCAGAGGTGAAATCTTGGATTTACGAAAGACGAACAACCTGCG	300		
Query 306	AAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGA	365		
Sbjct 301	AAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGA	360		
Query 366	TCAGATACCGTCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATT	425		
Sbjct 361	TCAGATACCGTCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATT	420		
Query 426	TTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTGGGTTCCGGGGGAAGTATGG	485		
Sbjct 421	TTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTGGGTTCCGGGGGAAGTATGG	480		
Query 486	TTGCAAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCAGGAGTGGAGCCTGCGG	545		
Sbjct 481	TTGCAAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCAGGAGTGGAGCCTGCGG	540		
Query 546	CTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGA-TGACAGAT	604		
Sbjct 541	CTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGAT	600		
Query 605	TGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGC	644		
Sbjct 601	TGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGC	640		

Figure 16: BLASTn search of Consensus 1 against Consensus 2. This figure shows the high identity of the 2 sequences.

Barneget Bay sequences generated from primer sets 2 and 3 were viewed and edited for accuracy as described above. Sequences were then aligned with consensus 1 to generate consensus 3, 1717 bp total (Fig. 17). When a BLASTn search was performed against consensus sequence 3, there was a score of ≥ 200 with a maximum identity of 98% (Fig. 18). A BLASTn match of consensus 3 against *Aurelia* sp. shows alignment from position 51-1764 bp (Fig. 19).

A.



B.

1-50	GGGCGGAGACGAAGGGAATGCTGGTCTAAGTAGAAGCACTAGCACATTGA
51-100	ACTGCGAATGGCTCGGAGAGAACCGGTTTATTTGATTGTACCTTACTACA
101-150	TGGATAACCGWGGTAATTCTAGAGCTAATACATGCGAAAAGTCCCGACTT
151-200	CTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTGCTTCGGTG
201-250	CCCGTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTG
251-300	CCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGG
301-350	TAGTGGCTTACCATGGTTACAACGGGTGACGGAGAATTAGGTTTCGATTC
351-400	CGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC
401-450	GCGCAAATTACCAATCCCGACACGGGGAGGTAGTGACAAGAAATAACAA
451-500	TCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGTACAATTTAAATCC
501-550	TTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAA
551-600	TTCCAGTCCAATAGCGTATATTAAGTTGTTGCAGTTAAAAAGCTCGTA
601-650	GTTGGATTTCCGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGC
651-700	TGGTCTGTCCTTCTTCGCAAAGACTGCGTGTGCTCTTAATTGAGTGTGCG
701-750	TAGGATTTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGT
751-800	GTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTTCTA
801-850	TTTTGTTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGG
851-900	CATTCGTATTTCTGTTGTCAGAGGTGAAATCCTTGGATTTACGAAAGACGA
901-950	ACAACCTGCGAAAGCATTGCGCAAGAATGTTTTCTTAATCAAGAACGAAA
951-1000	GTTAGAGGCTCGAAGACGATCAGATACCGTCCTAGTTCTAACCATAAACG
1001-1050	ATGCCGACTAGGGATCAGCGGGCGTTATTTATGACCCCGTTGGCACCTT
1051-1100	ATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAGCTG
1101-1150	AAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGC
1151-1200	TTAATTTGACTCAACACGGGAAACTCACCAGGTCCAGACATAGTAAGGA
1201-1250	TTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCC
1251-1300	GTTTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGA
1301-1350	CCTTATCCTGCTAAATAGTCACACGAATCTCGCTTCGTGCCTGACTTCTT
1351-1400	AGAGGGACTGTTGGTGTTAACCATCGTAAGGAAGGCAATAACAGGTCTGT
1401-1450	GATGCCCTTAGATGTTCTGGGCCGACGCGCTACACTGACGATGGCAG
1451-1500	CGAGTCTTAACCTTCGCCGATAGGTGTGGGTAACTTTGTCAAACATCGTC
1501-1550	GTGATGGGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTA
1551-1600	GTAAGCGCGAGTCATCAGCTCGCGTTGATTACGTCCTGCCCTTTGTACA
1601-1650	CACCGCCCGTCGCTACTACCGATTGAATGGTCTAGCGAGACCTTCGGATT
1651-1700	GGCACCGCTCTATAAGCAACGGCTGAGGTGGAACGCCGAGGAGTCGTCAA
1701-1717	GTCGATCATTAGAGGAA

Figure 17: Development of consensus sequence 3. A. Alignment of primer sets 1-3 Barnegat Bay sequences using Codon Code Aligner. Orange depicts reverse sequences; Blue depicts forward sequences. Difference in color within each sample depicts variation in nucleotides among sequences, which are regions with large peaks. Shows the overlap of the sequences to produce consensus sequence 3, shown in B.

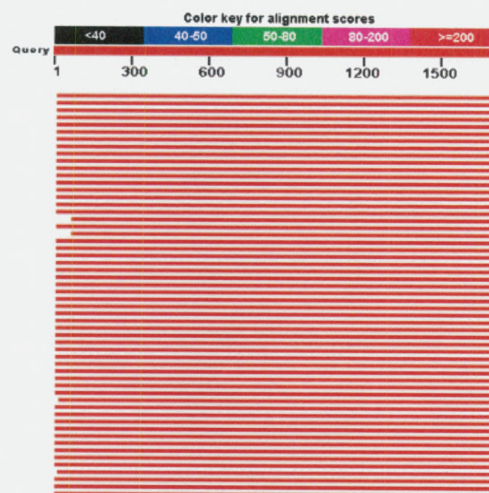
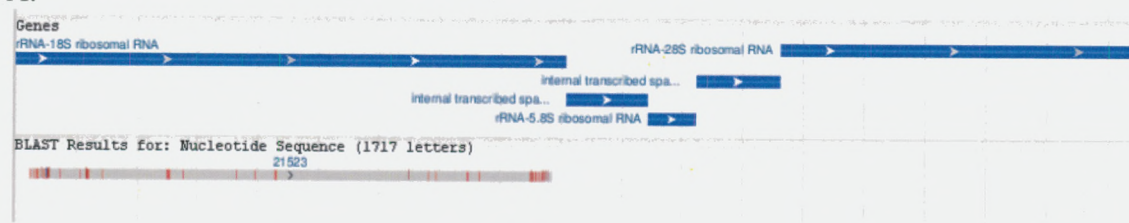


Figure 18: BLASTn matches to consensus sequence 3. The highest match is with *Chrysaora* sp. (AY920769.1) of 98% maximum identity.

A.



B.

Query	18	ATGCTGGTCTAAGTAGAAGCACTAGCACATTGAA-CTGCGAATGGCTCGGAGAGAACCG-	75
Sbjct	51	ATGCATGTCTAAGTATAAGCACTTGACTGTGAAACTGCGAATGGCTCATTAA-ATCAGT	109
Query	76	---GTTTATTGATTGATCTTACTACATGGATAACCGWGGTAATTCTAGAGCTAATAC	131
Sbjct	110	TATCGTTTATTGATTGATCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATAC	169
Query	132	ATGCGAAAAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGT	191
Sbjct	170	ATGCGAAAAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAGCCAATACGGGT	229
Query	192	GCT---TCGGTGCCCGTTTCATGTGGTGATTCATGATAACTTCTCGAATCGCATGGCCTT	247
Sbjct	230	GCTCTTTTGGGCACACGTTTCATGTGGTGATTCATGATAACTTCTCGAATCGCATGGCCTT	289
Query	248	GTGCCGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGC	307
Sbjct	290	GCGCCGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGC	349
Query	308	TTACCATGGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAG	367
Sbjct	350	TTACCATGGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAG	409
Query	368	AAACGGCTACCACATCCAAGGAAGGCAGGCGCGCAAATTACCCAATCCCGACACGGG	427
Sbjct	410	AAACGGCTACCACATCCAAGGAAGGCAGGCGCGCAAATTACCCAATCCCGACACGGG	469
Query	428	GAGGTAGTGACAAGAAATAACAATCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGT	487
Sbjct	470	GAGGTAGTGACAAGAAATAACAATCCGTGTCCATATTCTGGATGCGAAATTGGAATGAGT	529

Query	488	ACAATTTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG	547
Sbjct	530	ACAATTTAAATCCTTTAACGAGGACCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG	589
Query	548	TAATTCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGAT	607
Sbjct	590	TAATTCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGAT	649
Query	608	TTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCCTTCTTCG	667
Sbjct	650	TTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCCTTCTTCG	709
Query	668	CAAAGACTGCGTGTGCTCTTAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAA	727
Sbjct	710	CAAAGACTGCGTGTGCTCTTAAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAA	769
Query	728	AATTAGAGTGTTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAG	787
Sbjct	770	AATTAGAGTGTTCAAAGCAGGCTAACCCTTGAATACATGAGCATGGAATAATGGAATAG	829
Query	788	GACTTTGGTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGG	847
Sbjct	830	GACTTTGGTTCTATTTTGTGGTTTCTAGAACTGAAGTAATGATTAAGAGGGACAGTTGG	889
Query	848	GGGCATTTCGTATTTTCGTTGTGAGAGGTGAAATTTCTGGATTACGAAAGACGAACAACTG	907
Sbjct	890	GGGCATTTCGTATTTTCGTTGTGAGAGGTGAAATTTCTGGATTACGAAAGACGAACAACTG	949
Query	908	CGAAAGCATTTCGCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGAC	967
Sbjct	950	CGAAAGCATTTCGCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGAC	1009
Query	968	GATCAGATACCGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTA	1027
Sbjct	1010	GATCAGATACCGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTA	1069
Query	1028	TTTTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTAT	1087
Sbjct	1070	TTTTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTAT	1129
Query	1088	GGTTGCAAGAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGC	1147
Sbjct	1130	GGTTGCAAGAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGC	1189
Query	1148	GGCTTAATTTGACTCAACACGGGAAAACCTACCAGGTCCAGACATAGTAAGGATTGACAG	1207
Sbjct	1190	GGCTTAATTTGACTCAACACGGGAAAACCTACCAGGTCCAGACATAGTAAGGATTGACAG	1249
Query	1208	ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTT-TTAGTTGGTGGAG	1266
Sbjct	1250	ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAG	1309
Query	1267	TGATTTGTCTGGTTAATTCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGA	1326
Sbjct	1310	TGATTTGTCTGGTTAATTCGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGA	1369
Query	1327	ATCTCGCTTCGTGCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGC	1386
Sbjct	1370	ATCTCGCTTCGTGCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGC	1429
Query	1387	AATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGATG	1446
Sbjct	1430	AATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGATG	1489
Query	1447	GCAGCGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATG	1506
Sbjct	1490	TCAACGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATG	1549
Query	1507	GGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATC	1566
Sbjct	1550	GGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATC	1609
Query	1567	AGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGA	1626
Sbjct	1610	AGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGA	1669
Query	1627	ATGGTCTAGCGAGACCTTCGGATTGGCACCG-CTCTATAAGCAACGGCTGAGGTGGAACG	1685
Sbjct	1670	ATGGTCTAGCGAGACCTTCGGATTGGCGCCGTTTCGGCCCGCAAGGGCTGAAGTGGATAG	1729

```

Query 1686 CCGAGGAGTCG-TCAAG-TCGATCA-TTAGAGGAA 1717
          ||||| ||||| || || ||||| |||||
Sbjct 1730 CCGAGAAGTCGCTCTAGTTCGATCATTAGAGGAA 1764

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Figure 19: BLASTn results of consensus 3 against *Aurelia* sp. (EU276014.1). A. BLASTn graphics of consensus sequence 2 with *Aurelia* sp. where *Aurelia* sequences are indicated in blue; consensus 2 indicated in grey. Red regions demonstrate variability between sequences. B. Alignment of consensus 3 against *Aurelia*. This figure shows high identity with *Aurelia* matching 51-1764 bp.

Set 4 primers (5 and 8) were used to amplify the 5' end of the 28S rDNA sequence of approximately 1138 bp. Successful amplification was confirmed on 1% agarose gels. A band of approximately 1100 bp is present in all samples from Barnegat Bay, lanes 2-3, 5-6 are prominent clear bands; lane 4 is a very faint band (Fig. 20). In Lanes 7 and 8, no band of fragment size 1100 is seen for Chesapeake Bay and Navesink River samples, respectively. Lanes 2-6 were used for sequencing to determine 28S sequences.

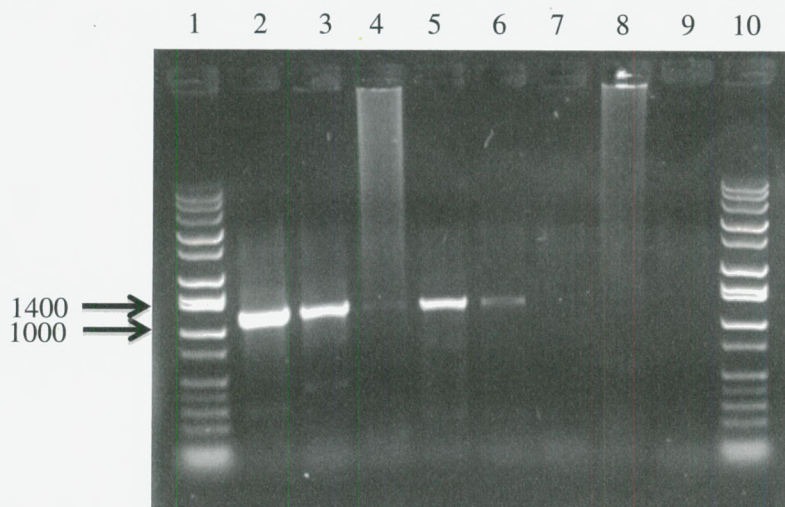


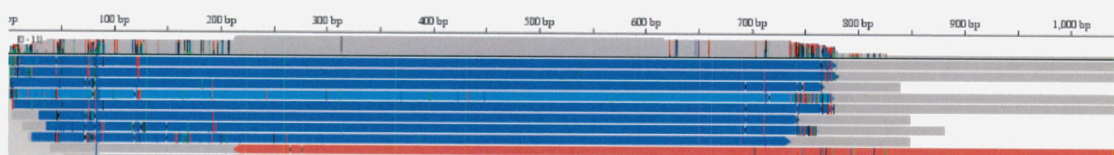
Figure 20: Agarose gel of Primer Set 4. Lane 1 = Hi/lo marker; Lanes 2-6 = Barnegat Bay CQ; Lane 7 = Chesapeake Bay CQ; Lane 8 = Navesink River CQ; Lane 9 = sterile deionized water (negative control); Lane 10 = Hi/lo marker. Lanes 2-6 were used for sequencing.

Sequencing reactions for primer set 4 were run and viewed as described above. Approximately 750 nts were generated from the forward primer sequences. Reverse primer sequences demonstrated poor quality, which made it difficult to identify

individual nucleotides. Quality for each reverse reaction are listed as follows: 1R=256nts; 2R=173nts, 3R=198nts, 4R=233nts, and 5R=139nts.

Reaction 4 was then re-sequenced in hope for better quality of the reverse primer sequences. New sequence quality data are as follows: 1R=359nts; 2R=580nts, 3 was not run, 4R=392nts, and 5R=152nts. While the second sequence reaction demonstrated an increase in quality, overall, quality was still very poor. The second sample, which had the highest quality data was the only sample further analyzed. This sample was aligned with the forward sequences to develop consensus sequence 4 of 1046 bp (Fig. 21).

A.



B.

1-50	CTAGRAATAGTACTAACAGGCTTCCTCAGTACGGCGAGTGAAGTGGAACA
51-100	GCTCAAGCTTGAAATCTCTGATGCTWGCAACACCGAATTGTAGTCTCGAG
101-150	AAGCGTCTTCCCGGTGGACCAGTCCTGTCTAAGTTGCTTGGAACAGCACA
151-200	TCGGAGAGGGTGACAATCCCGTACGCGACTGGACTGGCTGCCAACGATAC
201-250	GCTTCCATGAGTCGGGTTGCTTGGGAATGCAGCCCCAAAATTGGTGGTAA
251-300	ACTCCATCTAAAGCTAAATATTGGCAGGACCGATAGCGAACAAGTACC
301-350	GTGAGGGAAAGATGAAAAGAACTTTGAAAAGAGAGTTAAACAGTGCCTGA
351-400	AACCGTTAGAGGGGAAACGGATGCAGTTAGCAATGCTTCGGCGAGATTCA
401-450	GGCGATCGTTTCGCTGGACTTGCAGCGTAGGGATCCGAATGGACCGTCGC
451-500	TGTTCCGGCTCGGTGTTGTGTTTCGTCGCACTTCTCGTCGGAGCGCGTCAAC
501-550	AGCTGCTCTGTCTGGGCGAAAAGGCATCCAGGAAGGTAGGCGGGTACTTC
551-600	GGTACGTGCTGTTATAGCCTGTGGTGATACAAGCTCGGACGGGGCAGAGG
601-650	CGAACGGTGCATGCTTCTTTCGGAGGGGCTGGCGCCCTTCTTTGACAG
651-700	GATGTCGACCATGGCGGACTGCGTGCAAGTGCCTGAAATGCTTTTGTCT
701-750	ACTCGGGAGGATCGTGCACACATGCACTTTGGTTGTTGGCGGCCATATGG
751-800	CTTCATCCCTACCCCCCTGGAACACGGACCAAGGAGTATATCATGTGCGC
801-850	AAGTCTTGCGGTGATCGAATCCCAAAGGCACAATGAAAGTAAAGGCTGCC
851-900	TGGCAGCCGAGGTTAGATCTTCTGTTCTGCGCGTTCGAAGCGCATCATCGA
901-950	CCGACCTATTCTACTCTTAGAAAGGTTTGTAGTAAGAGCGCATCTGTTGGG
951-1000	ACCCGAAAGATGGTGAACATATGCTGAGTAGGGTGAAGCCAGAGGAAACT
1001-1046	AAAAGGAAGCTCGTAGCGATTCTGACGAGCAAATCGATCGTCAAC

Figure 21: Development of consensus sequence 4. A. Alignment of primer set 4 Barnegat Bay sequences using Codon Code Aligner. Orange depicts reverse sequence; Blue depicts forward sequences. Difference in color within each sample depicts variation in nucleotides among sequences, which show regions of large peaks. Shows the overlap of the sequences to produce consensus sequence 4, shown in B.

When a BLASTn search was performed against consensus sequence 4, results indicate a score of ≥ 200 with a maximum identity of 97% (Fig. 22). A BLASTn match of consensus 4 against *Aurelia* sp. shows alignment from position 2588-3625 bp with an 83% maximum identity (Fig. 23). Using *Aurelia* sp. as a reference where 28S is 3606 bp long (from position 2523-6128 in the 45S rDNA cassette), consensus 4 consists of $>28\%$ of 28S.



Figure 22: BLASTn matches to consensus sequence 4. The highest match is with *Chrysaora* sp. (AY920779.1) of 97% maximum identity.

Query	12	ACTAACA-GGCTTCC-TCAGTA-CGGCGAGTGAAGTGG-AACAGCTCAAGCTTGAAATCT	67
Sbjct	2588	ACTAACAAGGATTCCCTCAGTAACGGCGAGTGAAGTGGGAATGGCTCAAGCTTGAAATCT	2647
Query	68	CTGATGCTWCAACACCGAATTGTAGTCTCGAGAAGCGTCTTCCCGTGGACCACTCCTG	127
Sbjct	2648	CTGTTGCTTGCAACAGCGAATTGTAGTCTCGAGAAGCGTTTTCCCGCGGATCTATCCTG	2707
Query	128	TCTAAGTTGCTTGGAACAGCACATCGGAGAGGGTGACAATCCCGTACGCGACTGGACTGG	187
Sbjct	2708	CCTAAGTTGCTTGGAACAGCACATCAGAGAGGGTGACAATCCCGTTGTGGCAGGGCAGA	2767
Query	188	CTGCCAACGATACGCTTTCATGAGTCGGGTTGCTTGGGAATGCAGCCAAAATTGGTGG	247
Sbjct	2768	CCGTTTCATGATACGCTTTCATGAGTCGGGTTGCTTGGGAATGCAGCCAAAATTGGTGG	2827
Query	248	TAAATCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAAGTACCGTGAGGG	307
Sbjct	2828	TAAATCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAAGTACCGTGAGGG	2887
Query	308	AAAGATGAAAAGAACTTTGAAAAGAGAGTTAAACAGTGCCTGAAACCGTTAGAGGGGAAA	367
Sbjct	2888	AAAGATGAAAAGAACTTTGAAAAGAGAGTTAAAGAGTGCCTGAAACCGTTAGAGGGGAAAG	2947
Query	368	CGGATGCAGTTAGCAATGCTTCGGCGAGATTGAGGCGATCGTTTCGCTGGACTTGCAGCG	427
Sbjct	2948	CGGATGCAGTTAGCAATGCTTTTGCAGATTGAGGCGGTCGCTCGGCCAGACGTGCAGTT	3007


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Query 428 TAGGGATCCGAATGGACCGTCGCTGTTTCGGCTCGGTGTTGTTTCGTCGCACTTCTCGTC 487
      |||
Sbjct 3008 GATGGATCCGAATGGACTGTCGCTGTACGGCCGGGTCTTGTGCCCGTCGCACTTCTCGCT 3067

Query 488 GGAGCGCGTCAACAGCTGCTCTGTCTGGGCGAAAAGGCATCCA--GGAAGGTAGGCGGGT 545
      |||
Sbjct 3068 GGAGCGCGTCAACAGCTGCTGGGCTGGTCGACACGG--TCAAGTGAAGGTAAGCATCC 3125

Query 546 ACTTCGGTACGTGCTGTTATAGCCTGTGGTGATACAAGCTCGGACGGGGCAGAGGCGAAC 605
      |||
Sbjct 3126 AGCTTGCTGGCTGTTGTTATATCCACTCGT--ACCTAGATCGGGCTTGGCAGAGGCGAGC 3183

Query 606 GGTGCATGCT-TCTCTTCGGAGGGGCTGGCGCCCTCTTCTTGACAGGATGTCGACCATGG 664
      |||
Sbjct 3184 GGTGCATGCTCTCTC-TCGAGAGGGCTGGCGTCTGTTCCGTTACGTAGCCGGCTTGA 3242

Query 665 CGGACTGCGTGCAGTGC-CTGAAATGCTTTTGTCACTCGG-GAGGA-TC-GTGCACA 720
      |||
Sbjct 3243 TGCCTGCGTGCAGTACGTTCT---TGC---CGGTGAAACGGCGTGCAGTCAACGCACT 3295

Query 721 C-ATGCACTTTGGTTGTTGGCGGCCATATGGCTTCATCCCTACCCCCCTGGAACACGGAC 779
      |||
Sbjct 3296 CGATGCACCAATGGTTGTTGGCGGCCATATGGCTTTATCCGACCCGCTCTGAAACACGGAC 3355

Query 780 CAAGGAGTATATCATGTGCGCAAGTCTTGCGGTGATCGAATCCCAAAGGCACAATGAAAG 839
      |||
Sbjct 3356 CAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGACCGAAACCCAGAGGCGCAATGAAAG 3415

Query 840 TAAAGGCTGCCT--GGCAGCCGAGGTTAGATCTTCGTTCTGCGCGTTCGAAGCGCATCAT 897
      |||
Sbjct 3416 TGAAGGCTTCCTTGTGAGGCTGAGGTGAGATCTTGCTCTACGCGAGTGAGGCGCATCAT 3475

Query 898 CGACCGACCTATTCTACTCTTAGAAAGGTTTGAGTAAGAGCGCATCTGTTGGGACCCGAA 957
      |||
Sbjct 3476 CGACCGACCTATTCTACTCTTAGAAAGGTTTGAGTAAGAGCGTACATGTTGGGACCCGAA 3535

Query 958 AGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACT-AAAAGGAAGCTCGTAG 1016
      |||
Sbjct 3536 AGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGCTCGTAG 3595

Query 1017 CGATTCTGACGAGCAAATCGATCGTCGAAC 1046
      |||
Sbjct 3596 CGATTCTGACGTCGAAATCGATCGTCGAAC 3625

```

Figure 23: BLASTn results of consensus 4 against *Aurelia* sp. (EU276014.1). This figure shows high identity with *Aurelia* matching positions 2588-3625.

Design of New Primer Sets and Sequencing

A new primer set (#5) was designed to obtain the missing 50 nucleotides at the 3' end of the 18S rDNA by using primer 1, an internal 18S forward primer, and and ITS1 reverse primer. Primer set 5 should yield a product of 1479 bp.

Set 5 primers are:

Primer 1 (Forward) CTCGTAGTTGGATTTTCGGGA

Primer 12 (Reverse) CGCACGAGCCGAGTCCACCTTAGAAG

Three different annealing temperatures were used during PCR amplification to determine the best PCR conditions. Reaction #5 PCR conditions were: initial denaturation at 95°C for 1 min (one cycle); denaturation at 95°C for 15 sec, annealing at 54°C, 56°C, or 58°C for 20 sec, extension at 72°C for 70s (35 cycles); final extension at 72°C for 7 min (one cycle); soak at 4°C until removed. 1% agarose gels were run to confirm successful amplification.

Gel results indicate all three annealing temperatures worked, with an annealing temperature of 54°C demonstrating the cleanest bands. All Barnegat Bay samples (lanes 2-6) had a clear distinct band of approximately 1500 bp (Fig. 24). Chesapeake Bay and Navesink River samples (lanes 7 and 8) did not generate this band. Lanes 2-6 were used for sequencing.

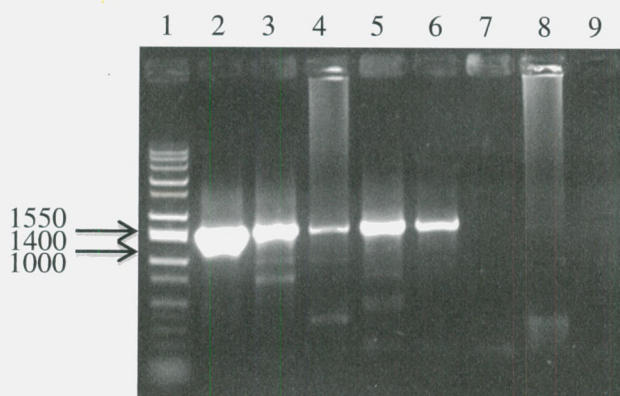
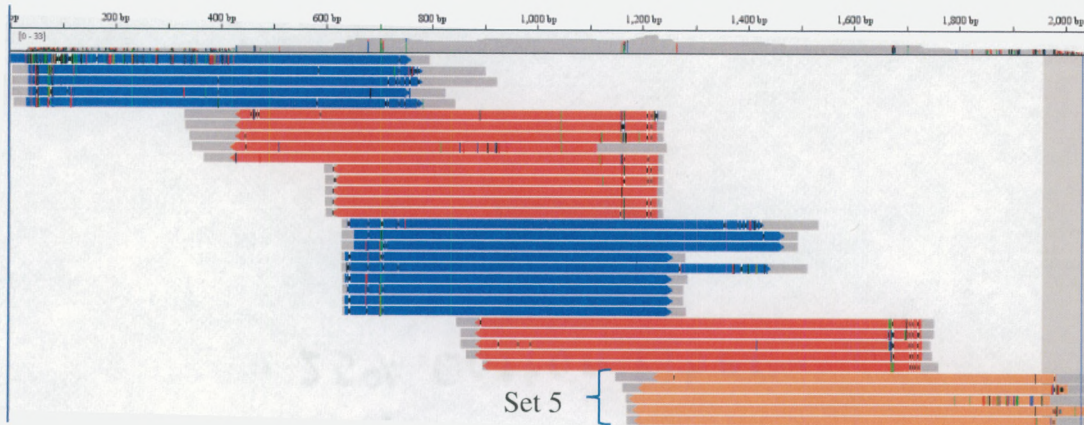


Figure 24: Agarose gel of Primer Set 5 (54°C). Lane 1 = Hi/lo marker; Lanes 2-6 = Barnegat Bay *CQ*; Lane 7 = Chesapeake Bay; Lane 8 = Navesink River; Lane 9 = sterile deionized water (negative control). Lanes 2-6 were used for sequencing reactions.

Sequencing reactions for primer set 5 were run and viewed as described above. Although some faint non-target bands were seen, clear electropherograms were obtained after being sequenced. Reverse sequences from set 5 were then aligned with consensus sequence 3 to produce consensus sequence 5, of 1943 bp (Fig. 25).

A.



B.

1-50	GGGCGGAGACGAAGGGAATGCTGGTCTAAGTAGAAGCACTAGCACATTGA
51-100	ACTGCGAATGGCTCGGAGAGAACCGGTTTATTTGATTGTACCTTACTACA
101-150	TGGATAACCGWGGTAATTCTAGAGCTAATACATGCGAAAAAGTCCCGACTT
151-200	CTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTGCTTCGGTG
201-250	CCCGTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTG
251-300	CCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGG
301-350	TAGTGGCTTACCATGGTTACAACGGGTGACGGAGAATTAGGGTTCGATTTC
351-400	CGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC
401-450	GCGCAAATTACCCAATCCCACACGGGGAGGTAGTGACAAGAAATAACAA
540-500	TCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGTACAATTTAAATCC
501-550	TTTAAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAA
551-600	TTCCAGCTCCAATAGCGTATATTTAAAGTTGTTGCAGTTAAAAAGCTCGTA
601-650	GTTGGATTTTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGC
651-700	TGGTCTGTCTTCTTCGCAAAGACTGCGTGTGCTCTTAATTGAGTGTGCG
701-750	TAGGATTTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCT
751-800	GTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTTCTA
801-850	TTTTGTTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGG
851-900	CATTCGTATTTTCGTTGTCAGAGGTGAAATTCCTGGATTACGAAAGACGA
901-950	ACAAGTGCAGAAAGCATTGGCCAAGAATGTTTTCTTAATCAAGAACGAAA
951-1000	GTTAGAGGCTCGAAGACGATCAGATACCGTCCTAGTTCTAACCATAAACG
1001-1050	ATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACCCCGTTGGCACCTT
1051-1100	ATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTG
1101-1150	AAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGC
1151-1200	TTAATTTGACTCAACACGGGAAAACCTACCAGGTCCAGACATAGTAAGGA
1201-1250	TTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCC
1251-1300	GTTCTTAGTTGGTGGAGTGATTGTCTGGTTAATTCCGTTAACGAACGAG
1301-1350	ACCTTATCCTGCTAAATAGTCACACGAATCTCGCTTCGTGCCTGACTTCT
1351-1400	TAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACAGGTCTG
1401-1450	TGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCA
1451-1500	GCGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGT
1501-1550	CGTGATGGGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCT
1551-1600	AGTAAGCGCGAGTCATCAGCTCGCGTTGATTACGTCCCTGCCCTTTGTAC
1601-1650	ACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGCGAGACCTTCGGAT
1651-1700	TGGCACCGCTCCGGCCGGAACCGGTGAGGTGGAACGCCGAGAAGTCGTT
1701-1750	CAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGTAGG
1751-1800	TGAACCTGCGGAAGGATCATTACCGAAAACGAGGCTAGCTCTTCCGAGAG
1801-1850	CATCGAATAGCTCTTGATCGACCGGGTCGTCGAAGGCTATGCGAACCCTG
1851-1900	TGAACCGTACCGATCTGTGTGAGGTGACCAGAGACAGGCCAGACTGGCA
1901-1943	GGCTGCAGTCGCTGCTGCTGGGCTTAAATGGCAGGCACTTTTC

Figure 25: Development of consensus sequence 5. A. Alignment of primer set 5 Barnegat Bay sequences with consensus sequence 3 using Codon Code Aligner. Orange

depicts reverse sequences (light orange indicates primer set 5 reverse sequences); Blue depicts forward sequences. Difference in color within each sample depicts variation in nucleotides among sequences, shows ranges with large peaks. Shows the overlap of the sequences used to produce Consensus sequence 4, shown in B. Black nucleotides indicate 18S rDNA (1772 nts); red nucleotides indicate ITS1 (170 nts). New forward primer (set #6) is underlined.

When a BLASTn search was performed against consensus sequence 5, results indicated a score of ≥ 200 with a maximum identity of 99% (Fig. 26). The highest match is with *Chrysaora* sp. (AY920769.1) of 99% maximum identity (Fig. 27). A BLASTn match of consensus 5 against *Aurelia* sp. shows alignment from position 57-1820 with a 97% identity and a 73% identity match from position 1895-2002 (Fig. 28). Set 5 primers successfully amplified the remaining nucleotides at the 3' end of 18S. Using *Aurelia* sp. as a reference where 18S is located at positions 1-1814 and ITS1 is located at positions 1815-2086 in the 45S rDNA cassette, consensus 5 includes $>96\%$ of the 18S rDNA sequence (1772 nts) and $>69\%$ of the ITS1 region (170 nts).

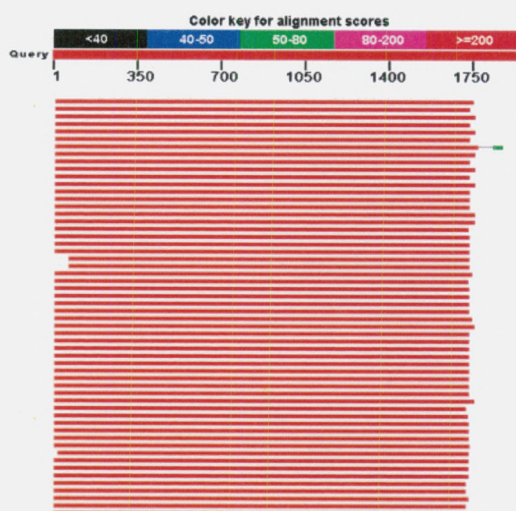


Figure 26: BLASTn matches to consensus sequence 5. The highest match is with *Chrysaora* sp. (AY920769.1) of 99% maximum identity.

Query	18	ATGCTGGTCTAAGTAGAAGCACTAGCACATTGAA-CTGCGAATGGCTCGGAGAGAACCG-	75
Sbjct	50	ATGCATGTCTAAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAA-ATCAGT	108
Query	76	---GTTTATTTGATTGTACCTTACTACATGGATAACCGWGGTAATTCTAGAGCTAATAC	131
Sbjct	109	TATCGTTTATTTGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATAC	168
Query	132	ATGCGAAAAGTCCCGACTTCTGGAAGGGATGTATTATTAGACTAAAAACCAATACGGGT	191
Sbjct	169	ATGCGAAAAGTCCCGACTTCTGGAAGGGATGTATTATTAGACTAAAAACCAATACGGGT	228
Query	192	GCTTCGGTGCCCGTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGC	251
Sbjct	229	GCCTCGGTGCCCGTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGC	288
Query	252	CGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTAC	311
Sbjct	289	CGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTAC	348
Query	312	CATGGTTACAACGGGTGACGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC	371
Sbjct	349	CATGGTTACAACGGGTGACGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC	408
Query	372	GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGG	431
Sbjct	409	GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGG	468
Query	432	TAGTGACAAGAAATAACAATCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGTACAA	491
Sbjct	469	TAGTGACAAGAAATAACAATCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGTACAA	528
Query	492	TTTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAAT	551
Sbjct	529	TTTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAAT	588
Query	552	TCCAGCTCCAATAGCGTATATTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCG	611
Sbjct	589	TCCAGCTCCAATAGCGTATATTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCG	648
Query	612	GGATGGGCCAGTCCGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTTCTTCGCAA	671
Sbjct	649	GGATGGGCCAGTCCGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTTCTTCGCAA	708
Query	672	GACTGCGTGTCTCTTAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAAAATT	731
Sbjct	709	GACTGCGTGTCTCTTAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAAAATT	768
Query	732	AGAGTGTTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACT	791
Sbjct	769	AGAGTGTTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACT	828
Query	792	TTGGTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGC	851
Sbjct	829	TTGGTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGC	888
Query	852	ATTTCGTATTTTCGTGTGTCAGAGGTGAAATTTCTGGATTACGAAAGACGAACAACCTGCGAA	911
Sbjct	889	ATTTCGTATTTTCGTGTGTCAGAGGTGAAATTTCTGGATTACGAAAGACGAACAACCTGCGAA	948
Query	912	AGCATTGCGCAAGAAATGTTTTCATTAAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATC	971
Sbjct	949	AGCATTGCGCAAGAAATGTTTTCATTAAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATC	1008
Query	972	AGATACCGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTT	1031
Sbjct	1009	AGATACCGTCCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTATTTT	1068
Query	1032	ATGACCCCGTTGGCACCTTATGGGAAACCAAGTTTTTGGGTTCCGGGGGAAGTATGGTT	1091
Sbjct	1069	ATGACCCCGTTGGCACCTTATGGGAAACCAAGTTTTTGGGTTCCGGGGGAAGTATGGTT	1128
Query	1092	GCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCT	1151
Sbjct	1129	GCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCT	1188
Query	1152	TAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTG	1211
Sbjct	1189	TAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTG	1248

Query	1212	AGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGAT	1271
Sbjct	1249	AGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGAT	1308
Query	1272	TTGTCTGGTTAATTCGGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCT	1331
Sbjct	1309	TTGTCTGGTTAATTCGGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCT	1368
Query	1332	CGCTTCGTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATA	1391
Sbjct	1369	CGCTTCGTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATA	1428
Query	1392	ACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAG	1451
Sbjct	1429	ACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCAG	1488
Query	1452	CGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGA	1511
Sbjct	1489	CGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATGGGGA	1548
Query	1512	TAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCT	1571
Sbjct	1549	TAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCT	1608
Query	1572	CGCGTTGATTACGTCCTGCCCCTTGTACACACGCCCGTCGCTACTACCGATTGAATGG	1631
Sbjct	1609	CGCGTTGATTACGTCCTGCCCCTTGTACACACGCCCGTCGCTACTACCGATTGAATGG	1668
Query	1632	TCTAGCGAGACCTTCGGATTGGCACCGCTCCGGCCGGCAACGGCTGAGGTGGAACGCCGA	1691
Sbjct	1669	TCTAGCGAGACCTTCGGATTGGCACCGCTCCGGCCGGCAACGGCTGAGGTGGAACGCCGA	1728
Query	1692	GAAGTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAAGTCGTAAACAGGTTTCCGTAGGT	1751
Sbjct	1729	GAAGTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAAGTCGTAAACAGGTTTCCGTAGGT	1788
Query	1752	GAACCTGC	1759
Sbjct	1789	GAACCTGC	1796

Figure 27: BLASTn match of consensus 5 against *Chrysaora* sp. (AY920769.1) with a 99% identity match.

A.

Query	1	GTCTAAGTATAAGCACTCG-ACGTTGAA-CTGCGAATGGCTCAC-AAAGRAATC--CGTT	55
Sbjct	57	GTCTAAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAATCAGTTATCGTT	116
Query	56	TATTTGATTGTACCTTACTACATGGATAACCGWGGTAATTCTAGAGCTAATACATGCGAA	115
Sbjct	117	TATTTGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGAA	176
Query	116	AAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTGCT----	171
Sbjct	177	AAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAGCCAATACGGGTGCTCTTT	236
Query	172	TCGGTGCCCGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGG	231
Sbjct	237	TGGGCACACGTTTCATGTGGTGATTTCATGATAACTTCTCGAATCGCATGGCCTTGTGCCGG	296
Query	232	CGATGTTTCATTCAAATTTCTGCCCTATCAACTGTGATGGTAAGGTAGTGGCTTACCAT	291
Sbjct	297	CGATGTTTCATTCAAATTTCTGCCCTATCAACTGTGATGGTAAGGTAGTGGCTTACCAT	356
Query	292	GGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGC	351
Sbjct	357	GGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGC	416
Query	352	TACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAG	411
Sbjct	417	TACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAG	476
Query	412	TGACAAGAAATAACAATCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGTACAATTT	471
Sbjct	477	TGACAAGAAATAACAATCCGTGTCCATATTCTGGATGCGAAATTGGAATGAGTACAATTT	536
Query	472	AAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATTCC	531
Sbjct	537	AAATCCTTTAACGAGGACCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATTCC	596

Query	532	AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGA	591
Sbjct	597	AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGA	656
Query	592	TGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCCCTTCTTCGCAAAGAC	651
Sbjct	657	TGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCCCTTCTTCGCAAAGAC	716
Query	652	TGCGTGTGCTCTTGATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAAAATTAGA	711
Sbjct	717	TGCGTGTGCTCTTAAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAAAATTAGA	776
Query	712	GTGTTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTG	771
Sbjct	777	GTGTTCAAAGCAGGCTAACCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTG	836
Query	772	GTTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGGCATT	831
Sbjct	837	GTTCTATTTTGTGGTTTCTAGAACTGAAGTAATGATTAAGAGGGACAGTTGGGGGCATT	896
Query	832	CGTATTTCTGTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGC	891
Sbjct	897	CGTATTTCTGTGTGAGAGGTGAAATCTTGGATTACGAAAGACGAACAACCTGCGAAAGC	956
Query	892	ATTTGCCAAGAATGTTTTCTTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGA	951
Sbjct	957	ATTTGCCAAGAATGTTTTCTTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGA	1016
Query	952	TACCGTCCTAGTTCTAACCATAAACGATGCCGACTAGGATCAGCGGGCGTTATTTTATG	1011
Sbjct	1017	TACCGTCCTAGTTCTAACCATAAACGATGCCGACTAGGATCAGCGGGCGTTATTTTATG	1076
Query	1012	ACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCA	1071
Sbjct	1077	ACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCA	1136
Query	1072	AAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA	1131
Sbjct	1137	AAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA	1196
Query	1132	TTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGA	1191
Sbjct	1197	TTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGA	1256
Query	1192	GCTCTTTCTTGATTCTATGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTG	1251
Sbjct	1257	GCTCTTTCTTGATTCTATGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTG	1316
Query	1252	TCTGGTTAATTCGGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGC	1311
Sbjct	1317	TCTGGTTAATTCGGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGAATCTCGC	1376
Query	1312	TTCGTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACA	1371
Sbjct	1377	TTCGTGCCTGGCTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGCAATAACA	1436
Query	1372	GGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGATGGCAGCGA	1431
Sbjct	1437	GGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGATGTCAACGA	1496
Query	1432	GTCTTAACCTTCGCCGATAGGTGTGGTAATCTTGTCAAACATCGTCGTGATGGGGATAG	1491
Sbjct	1497	GTCTTAACCTTCGCCGATAGGTGTGGTAATCTTGTCAAACATCGTCGTGATGGGGATAG	1556
Query	1492	ATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGC	1551
Sbjct	1557	ATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGC	1616
Query	1552	GTTGATTACGTCCCTGCCCTTTGTACACACCGCCGTCGCTACTACCGATTGAATGGTCT	1611
Sbjct	1617	GTTGATTACGTCCCTGCCCTTTGTACACACCGCCGTCGCTACTACCGATTGAATGGTCT	1676
Query	1612	AGCGAGACCTTCGGATTGGCACCCTCCGGCCGGCAACGGCTGAGGTGGAACGCCGAGAA	1671
Sbjct	1677	AGCGAGACCTTCGGATTGGCACCCTTCGGCCGGCAAGGGCTGAAGTGGATAGCCGAGAA	1736
Query	1672	GTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGTAGGTGAA	1731
Sbjct	1737	GTCGCTCTAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGTAGGTGAA	1796

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Query 1732 CCTGCGGAAGGATCATTACCGAAA 1755
          |||
Sbjct 1797 CCTGCGGAAGGATCATTACCGAAA 1820

```

B.

```

Query 1820 AACCACTGTGAACCCGTACCGATCTGTGTGAGGTGACCAGAGACAGGCCAGACTGGCAGG 1879
          |||
Sbjct 1895 AACCACTGTGAACCTGTACCTATCTGTGTGAGGTGGGCAGAG---TGC---ACATGC-GT 1947

Query 1880 CTGCAGTCGCTGTCGTCTGGGCTTAAATGGCAGGCACCTTTCCTGTCGGCCTCACATGGAG 1939
          |||
Sbjct 1948 GTGTA-----CGTCTGAGCTTAAATGACGGGCGTTTCCCATCGGCCTCACATGGAG 1999

Query 1940 TTT 1942
          |||
Sbjct 2000 TTT 2002

```

Figure 28: BLASTn match of Consensus 5 against *Aurelia* sp. 18S ribosomal RNA gene and internal transcribed spacer 1 (gbEU276014.1). A. Range 1(18S) shows that at the nucleotide level, there is 97% identity. B. Range 2(ITS1) demonstrates 73% identity.

Using consensus sequence 4 (28S) and 5 (18S, ITS1) data, the next step was to design a primer set that could close the gap between 18S and 28S, to amplify the missing ITS1 nts, 5.8S, and ITS2 regions. Using consensus sequences 4 and 5, a new primer set (#6) was designed. Primer 13 (forward) was designed by finding a conserved region at the 3' end of 18S in consensus 5 (see Fig. 25). Primer 14 (reverse) was designed at the 5' end of the 28S sequence. This was done by taking the reverse complement of primer 5, which was a forward primer used in primer set 4. Two nucleotides were added to this sequence for a higher melting temperature. Designed primers were analyzed as described above. By aligning each primer with *Aurelia* sp., it is predicted that primer set 6 should yield a product of approximately 775 bp (Fig. 29).

Set 6 primers are:

Primer 13 (18S Forward) TAGGTGAACCTGCGGAAGGA

Primer 14 (28S Reverse) TATGCTTAAATTCAGCGGGTAG

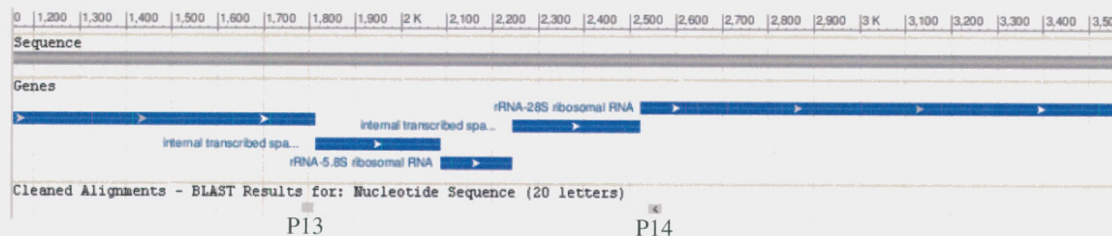


Figure 29: BLASTn Graphics of primer set 6 against *Aurelia* sp. (gbEU276014.1) to predict possible amplification length. Blue depicts *Aurelia* sequences. Grey depicts primer set 6 locations.

Two annealing temperatures were run to indicate optimal reaction conditions during amplification. Reaction #6 conditions were: initial denaturation at 95°C for 1 min (one cycle); denaturation at 95°C for 15 sec, annealing at 50 or 51°C for 20 sec, extension at 72°C for 48 sec (35 cycles); final extension at 72°C for 7 min (one cycle); soak at 4°C until removed. 1% agarose gels were run to confirm successful amplification. A band of approximately 700 bp was seen in all Barnegat Bay and Navesink River samples (lanes 2-6, 8) (Fig. 30). No bands are present in the Chesapeake Bay sample (lane 7). Samples from lanes 2-6 and 8 were used for sequencing.

Sequencing reactions for primer set 6 were run and viewed as described above. From set 6 sequence results, two consensus sequences were generated: one from Barnegat Bay samples and one from Navesink River. 660 bp were generated from set 6 Barnegat Bay samples to create consensus sequence 6 (Fig. 31). Navesink River forward and reverse sequences were assembled to create consensus sequence 7, a sequence of 726 bp total (Fig. 32). Consensus sequence 7 is 66 nts longer than consensus 6 due to higher quality results, and therefore there is more data at both the 5' and 3' end of the sequence.

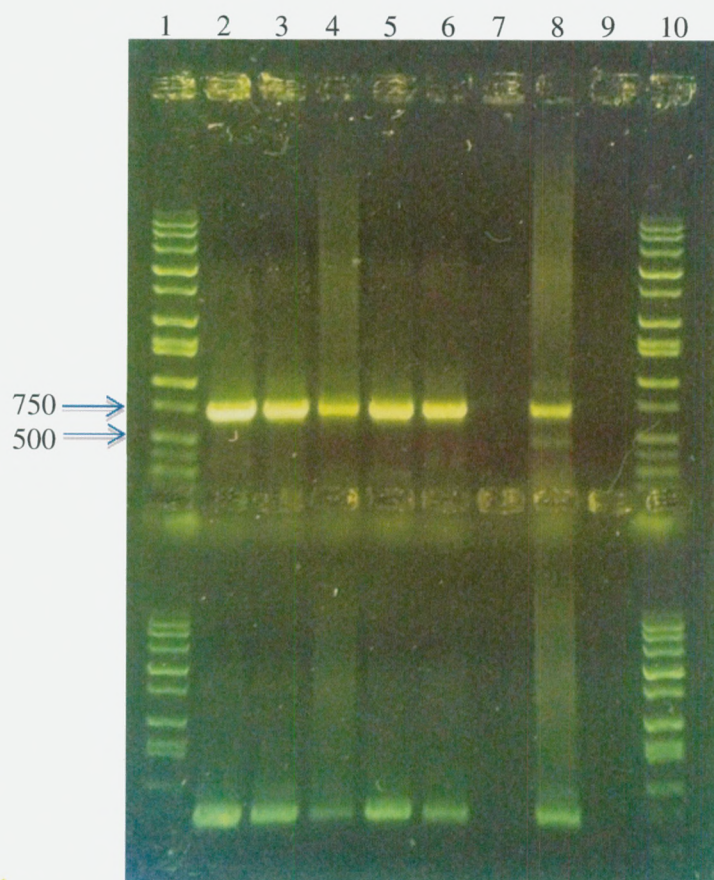


Figure 30: Agarose gel of Primer Set 6. Top and bottom lanes have an annealing temperature of 50°C and 51°C, respectively. Lane 1 = Hi/lo marker; Lanes 2-6 = Barnegat Bay CQ; Lane 7 = Chesapeake Bay; Lane 8 = Navesink River; Lane 9 = sterile deionized water (negative control). Lanes 2-6 and 8 were used for sequence reactions.

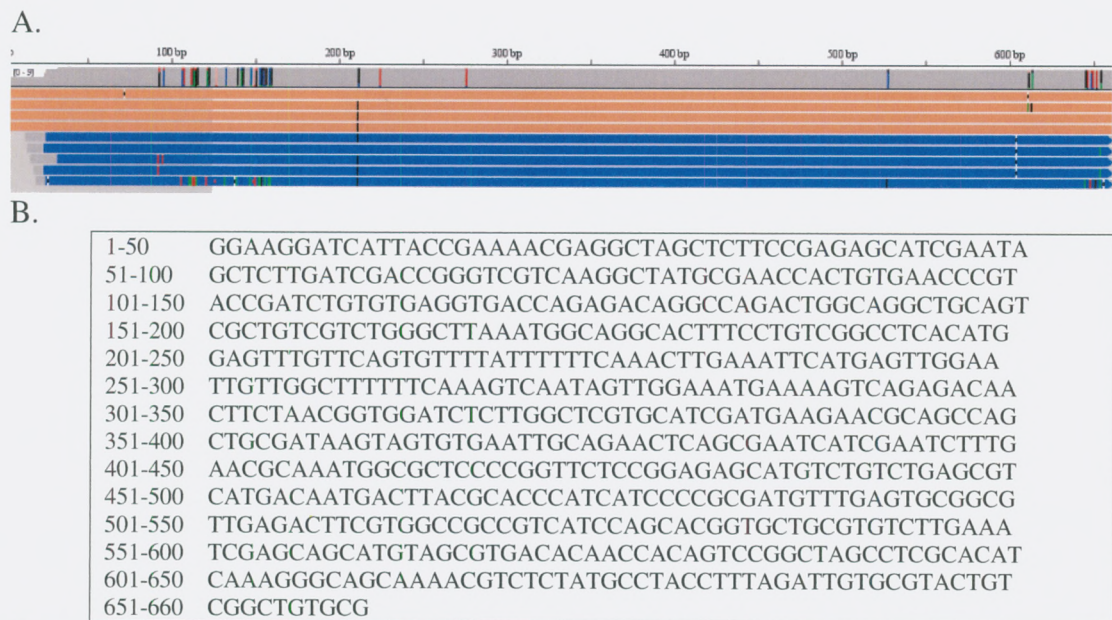


Figure 31: Development of consensus sequence 6. A. Alignment of primer set 6 Barnegat Bay sequences using Codon Code Aligner. Orange depicts reverse sequences; blue depicts forward sequences. Difference in color depicts variation in nucleotides among sequences. Shows the overlap of the sequences used to produce consensus sequence 6, shown in B.

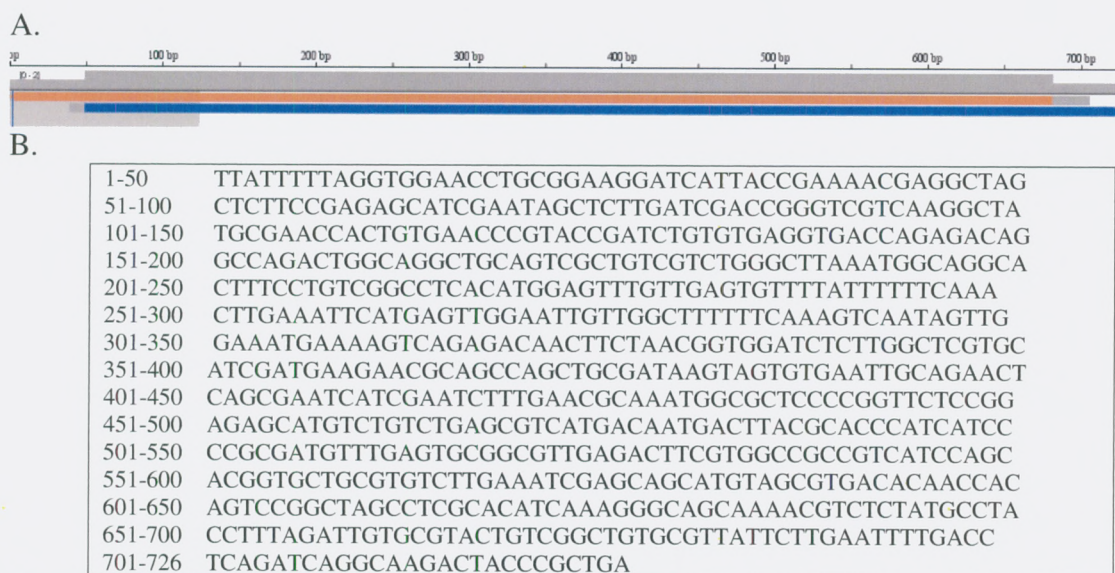


Figure 32: Development of consensus sequence 7. A. Alignment of primer set 6 Navesink River sequences using Codon Code Aligner. Orange depicts reverse sequence; blue depicts forward sequence. Shows the overlap of the sequences used to produce consensus sequence 7, shown in B.

Upon a BLASTn search against consensus 6, results indicated a score of ≥ 200 for most samples with an 80% maximum identity (Fig. 33). Some sequences yielded a much lower alignment score in some regions as little as 40-50. Search results indicate consensus 6 includes sequences from ITS1, 5.8S, and ITS2, confirming successful amplification. Results for consensus 7 were similar when a BLASTn search was performed (Fig. 34). However, alignment scored ranges from as high at ≥ 200 to as little as 40-50 in some regions. This is to be expected since ITS regions are variable among species. When a BLASTn search of consensus 6 against consensus 7 was performed, results indicated high identity, a 99% match (Fig. 35). Only one nt differed between sequences at position 210.

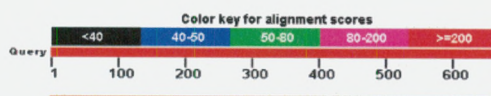


Figure 33: BLASTn matches of consensus sequence 6.



Figure 34: BLASTn matches of consensus sequence 7.

A.



B.

Score	Expect	Identities	Gaps	Strand
1186 bits(1314)	0.0	659/660(99%)	0/660(0%)	Plus/Plus
Query 1	GGAAAGGATCATTACCGAAAAACGAGGCTAGCTCTTCCGAGAGCATCGAATAGCTCTTGATC	60		
Sbjct 22	GGAAAGGATCATTACCGAAAAACGAGGCTAGCTCTTCCGAGAGCATCGAATAGCTCTTGATC	81		
Query 61	GACCGGGTCGTCAAGGCTATGCGAACCACTGTGAACCCGTACCGATCTGTGTGAGGTGAC	120		
Sbjct 82	GACCGGGTCGTCAAGGCTATGCGAACCACTGTGAACCCGTACCGATCTGTGTGAGGTGAC	141		
Query 121	CAGAGACAGGCCAGACTGGCAGGCTGCAGTCGCTGTCGTCTGGGCTTAAATGGCAGGCAC	180		
Sbjct 142	CAGAGACAGGCCAGACTGGCAGGCTGCAGTCGCTGTCGTCTGGGCTTAAATGGCAGGCAC	201		
Query 181	TTTCCTGTCGGCCTCACATGGAGTTTGTTCAGTGTTTTATTTTTTCAAACCTGAAATTCA	240		
Sbjct 202	TTTCCTGTCGGCCTCACATGGAGTTTGTTCAGTGTTTTATTTTTTCAAACCTGAAATTCA	261		
Query 241	TGAGTTGGAATTGTTGGCTTTTTTCAAAGTCAATAGTTGGAATGAAAGTCAGAGACAA	300		
Sbjct 262	TGAGTTGGAATTGTTGGCTTTTTTCAAAGTCAATAGTTGGAATGAAAGTCAGAGACAA	321		
Query 301	CTTCTAACGGTGGATCTCTTGGCTCGTGCATCGATGAAGAACGACCCAGCTGCGATAAG	360		
Sbjct 322	CTTCTAACGGTGGATCTCTTGGCTCGTGCATCGATGAAGAACGACCCAGCTGCGATAAG	381		
Query 361	TAGTGTGAATTGCAGAACTCAGCGAATCATCGAATCTTTGAACGCAATGGCGCTCCCG	420		
Sbjct 382	TAGTGTGAATTGCAGAACTCAGCGAATCATCGAATCTTTGAACGCAATGGCGCTCCCG	441		
Query 421	GTTCTCCGGAGAGCATGTCTGTCTGAGCGTCATGACAATGACTTACGCACCCATCATCCC	480		
Sbjct 442	GTTCTCCGGAGAGCATGTCTGTCTGAGCGTCATGACAATGACTTACGCACCCATCATCCC	501		
Query 481	CGCGATGTTTGAAGTGGCGGTTGAGACTTCGTGGCCGCCGTATCCAGCACGGTGTGCG	540		
Sbjct 502	CGCGATGTTTGAAGTGGCGGTTGAGACTTCGTGGCCGCCGTATCCAGCACGGTGTGCG	561		
Query 541	TGCTTTGAAATCGAGCAGCATGTAGCGTGACACAACACAGTCCGGGTAGCCTCGCAT	600		
Sbjct 562	TGCTTTGAAATCGAGCAGCATGTAGCGTGACACAACACAGTCCGGGTAGCCTCGCAT	621		
Query 601	CAAAAGGACAGCAAAACGCTCTATGCCTACCTTTAGATTGTGCGTACTGTGCGGTGTGCG	660		
Sbjct 622	CAAAAGGACAGCAAAACGCTCTATGCCTACCTTTAGATTGTGCGTACTGTGCGGTGTGCG	681		

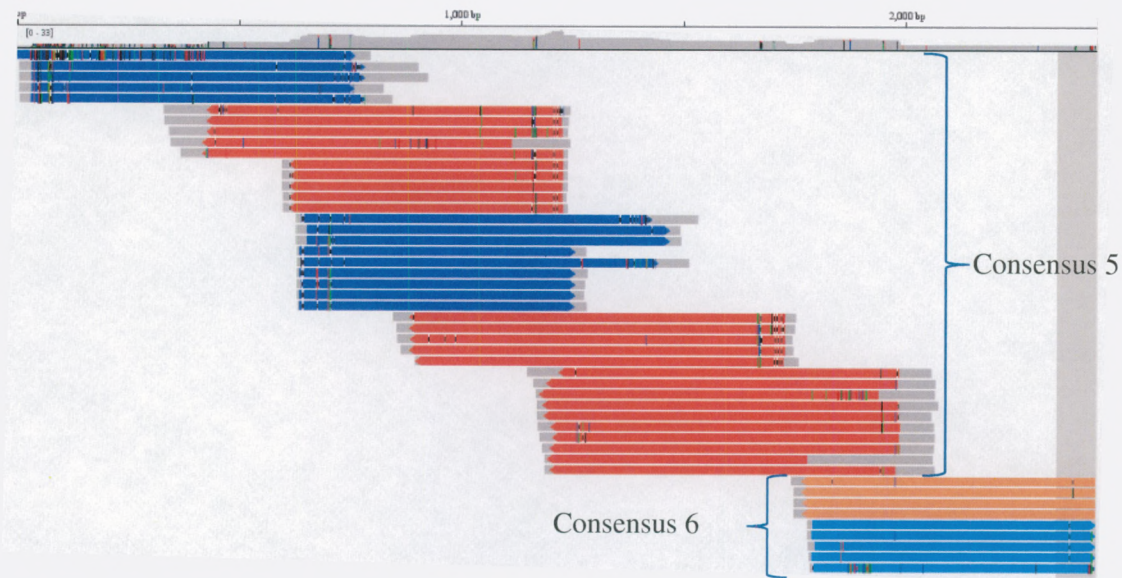
Figure 35: BLASTn search of Consensus 6 against Consensus 7. This figure shows the high identity of the two sequences.

Final Contig Development

A final consensus sequence was created by aligning all Barnegat Bay samples from reaction sets 1-6. Contig1a was created by assembling together consensus 5 and 6, a final sequence of 2419 bp, covering 18S, ITS1, 5.8S, and ITS2 regions (Fig. 36). Contig1b includes data from consensus 4, a final sequence of 1046 bp covering 28S.

A BLASTn search was performed against Contig1 to search for nucleotide matches against any organism (Fig. 37). Contig1 had a score of ≥ 200 . The highest match was with *Aurelia* sp., a maximum identity of 92%. By aligning *Aurelia* with Contig 1a and 1b, the position of nts were identified. In Contig1a, position 1-1772 (1772 nts total) represents 18S with a 47.1% GC content, 1773-2056 (284 nts total) is ITS1 with a 47.9% GC content, 2057-2214 (158 nts total) is 5.8S with a 51.3% GC content, and 2215-2419 (205 nts total) is ITS2 with a 56.1% GC content. In Contig1b, 1-1046 (1046 nts total) represents 28S with a 52.5% GC content (Fig. 36-37). By comparing Contig 1a and 1b to *Aurelia*, there is a gap between Contig 1a and 1b of approximately 65 bp at the 5' end of 28S.

A.



B.

1-50	GGGCGGAGACGAAGGGAATGCTGGTCTAAGTAGAAGCACTAGCACATTGA
51-100	ACTGCGAATGGCTCGGAGAGAACCGGTTTATTTGATTGTACCTTACTACA
101-150	TGGATAACCGWGGTAATTCTAGAGCTAATACATGCGAAAAGTCCCGACTT
151-200	CTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTGCTTCGGTG
201-250	CCCGTTCATGTGGTGATTCATGATAACTTCTCGAATCGCATGGCCTTG
251-300	CCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGG
301-350	TAGTGGCTTACCATGGTTACAACGGGTGACGGAGAATTAGGGTTTCGATT
351-400	CGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC
401-450	GCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAAGAAATAACAA
451-500	TCCGTGTCTATTTATTAGACGCGAAAATTGGAATGAGTACAATTTAAATCC
501-550	TTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAA
551-600	TTCCAGCTCCAATAGCGTATATTTAAAGTTGTTGCAGTTAAAAAGCTCGTA
601-650	GTTGGATTTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGC
651-700	TGGTCTGTCTTCTTCGCAAAGACTGCGTGTGCTCTTAATTGAGTGTGCG
701-750	TAGGATTTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCT
751-800	GTCCGCTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTTCTA
801-850	TTTTGTTGGTTTCTGGAACCGAAGTAATGATTAAAGAGGGACAGTTGGGGG
851-900	CATTCGTATTTCTGTTGTCAGAGGTGAAATCTTGGATTTACGAAAGACGA
901-950	ACAACCTGCGAAAGCATTGCGCAAGAATGTTTTCAATTAATCAAGAACGAAA
951-1000	GTTAGAGGCTCGAAGACGATCAGATACCGTCCTAGTTCTAACCATAAACG
1001-1050	ATGCCGACTAGGGATCAGCGGGCGTTATTTTATGACCCCGTTGGCACCTT
1051-1100	ATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTG
1101-1150	AAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGC
1151-1200	TTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGA
1201-1250	TTGACAGATTGAGAGCTCTTCTTGATTCTATGGGTGGTGGTGATGGCC
1251-1300	GTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTAAACGAACGAG
1301-1350	ACCTTATCCTGCTAAATAGTCACACGAATCTCGTTCGTGCCTGACTTCT
1351-1400	TAGAGGGACTGTTGGTGTTAACCATCGTAAGGAAGGCAATAACAGGTCTG
1401-1450	TGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGATGGCA
1451-1500	GCGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGT
1501-1550	CGTGATGGGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCT
1551-1600	AGTAAGCGCGAGTCATCAGCTCGCGTTGATTACGTCCCTGCCCTTTGTAC
1601-1650	ACACCGCCCGTCGCTACTACCGATTGAATGGTCTAGCGAGACCTTCGGAT
1651-1700	TGGCACCGCTCCGGCCGGCAACGGCTGAGGTGGAACGCCGAGAAGTCGTT
1701-1750	CAAGTTCGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGG
1751-1800	TGAACCTGCGGAAGGATCATTACCGAAAACGAGGCTAGCTCTTCCGAGAG
1801-1850	CATCGAATAGCTCTTGATCGACCGGGTCGTCAAGGCTATGCGAACCCTG

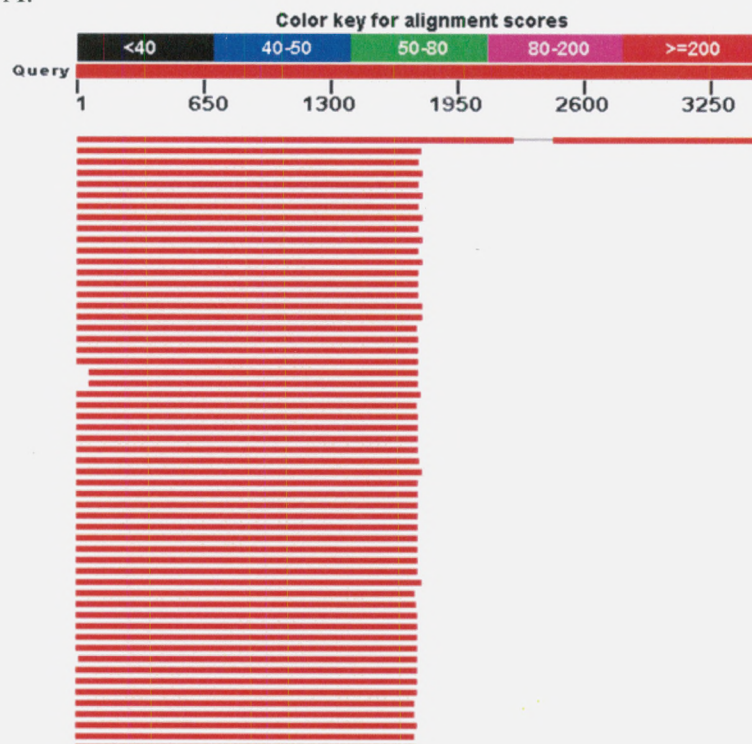
1851-1900	TGAACCCGTACCGATCTGTGTGAGGTGACCAGAGACAGGCCAGACTGGCA
1901-1950	GGCTGCAGTCGCTGTCGTCTGGGCTTAAATGGCAGGCACTTTCTGTCCG
1951-2000	CCTCACATGGAGTTTGTGTGAGTGTATTTTCAAACCTTGAAATTCAT
2001-2050	GAGTTGGAATTGTGGCTTTTTCAAAGTCAATAGTTGGAATGAAAAGT
2051-2100	CAGAGACAACCTTCTAACGGTGGATCTCTTGGCTCGTGCATCGATGAAGAA
2101-2150	CGCAGCCAGCTGCGATAAGTAGTGTGAATTGCAGAACTCAGCGAATCATC
2151-2200	GAATCTTTGAACGCAAATGGCGCTCCCCGGTTCTCCGGAGAGCATGTCTG
2201-2250	TCTGAGCGTCATGACAATGACTTACGCACCCATCATCCCCGCGATGTTG
2251-2300	AGTGCGGCGTTGAGACTTCGTGGCCGCCGTCATCCAGCACGGTGCTGCGT
2301-2350	GTCTTGAAATCGAGCAGCATGTAGCGTGACACAACCACAGTCCGGCTAGC
2351-2400	CTCGCACATCAAAGGGCAGCAAAACGTCTCTATGCCTACCTTTAGATTGT
2401-2419	GCGTACTGTCCGCTGTGCG

C.

1-50	CTAGRAATAGTACTAACAGGCTTCCTCAGTACGGCGAGTGAAGTGGAACA
51-100	GCTCAAGCTTGAAATCTCTGATGCTWGC AACACCCGAATTGTAGTCTCGAG
101-150	AAGCGTCTTCCCGTGGACCAAGTCCTGTCTAAGTTGCTTGGAACAGCACA
151-200	TCGGAGAGGGTGACAATCCCGTACGCGACTGGACTGGCTGCCAACGATAC
201-250	GCTTTCCATGAGTCGGGTTGCTTGGGAATGCAGCCCAAAATTGGTGGTAA
251-300	ACTCCATCTAAAGCTAAATATTGGCACGAGACCGATAGCGAACAAGTACC
301-350	GTGAGGGAAAGATGAAAAGAACTTTGAAAAGAGAGTTAAACAGTGCGTGA
351-400	AACCGTTAGAGGGGAAACGGATGCAGTTAGCAATGCTTCGGCGAGATTCA
401-450	GGCGATCGTTTCGCTGGACTTGCAGCGTAGGGATCCGAATGGACCGTCGC
451-500	TGTTCCGGCTCGGTGTTGTGTTCTGTCGCACTTCTCGTCGGAGCGCGTCAAC
501-550	AGCTGCTCTGTCTGGGCGAAAAGGCATCCAGGAAGGTAGGCGGGTACTTC
551-600	GGTACGTGCTGTTATAGCCTGTGGTGATACAAGCTCGGACGGGGCAGAGG
601-650	CGAACGGTGCATGCTTCTCTTCGGAGGGGCTGGCGCCCTCTTCTTGACAG
651-700	GATGTCGACCATGGCGGACTGCGTGCAGTGCCTGCTGAAATGCTTTTTGTC
701-750	ACTCGGGAGGATCGTGCACACATGCACTTTGGTTGTTGGCGGCCATATGG
751-800	CTTCATCCCTACCCCCCTGGAACACGGACCAAGGAGTATATCATGTGCGC
801-850	AAGTCTTGCGGTGATCGAATCCCAAAGGCACAATGAAAGTAAAGGCTGCC
851-900	TGGCAGCCGAGGTTAGATCTTCGTTCTGCGCGTTCGAAGCGCATCATCGA
901-950	CCGACCTATTCTACTCTTAGAAAAGGTTTGAGTAAGAGCGCATCTGTTGGG
951-1000	ACCCGAAAGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACT
1001-1046	AAAAGGAAGCTCGTAGCGATTCTGACGAGCAAAATCGATCGTCAAC

Figure 36: Development of Contig1a and 1b. A. Alignment of consensus 6 Barnegat Bay sequences with consensus 5 using Codon Code Aligner. Orange depicts reverse sequences; Blue depicts forward sequences. Difference in color within each sample depicts variation in nucleotides among sequences, shows range of large peaks. Shows the overlap of the sequences used to produce Contig1a, shown in B. Black denotes 18S (1772 nts), red denotes ITS1 (284 nts), blue denotes 5.8S (158 nts), green denotes ITS2 (205 nts). Contig1b depicts 28S, shown in C.

A.



B.

Range 1:

Query	18	ATGCTGGTCTAAGTAGAAGCACTAGCACATTGAA-CTGCGAATGGCTCGGAGAGAACCG-	75
Sbjct	51	ATGCATGTCTAAGTATAAGCACTTGTACTGTGAAACTGCGAATGGCTCATTAA-ATCAGT	109
Query	76	----GTTTATTGATTGTACCTTACTACATGGATAACCGWGGTAATTCTAGAGCTAATAC	131
Sbjct	110	TATCGTTTATTGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATAC	169
Query	132	ATGCGAAAAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGT	191
Sbjct	170	ATGCGAAAAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAGCCAATACGGGT	229
Query	192	GCT----TCGGTGCCCCGTTTCATGTGGTGATTCATGATAACTTCTCGAATCGCATGGCCTT	247
Sbjct	230	GCTCTTTTGGGCACACGTTTCATGTGGTGATTCATGATAACTTCTCGAATCGCATGGCCTT	289
Query	248	GTGCCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGC	307
Sbjct	290	GCGCCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGC	349
Query	308	TTACCATGGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAG	367
Sbjct	350	TTACCATGGTTACAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAG	409
Query	368	AAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGG	427
Sbjct	410	AAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGG	469
Query	428	GAGGTAGTGACAAGAAATAACAATCCGTGTCTATTTATTAGACGCGAAATTGGAATGAGT	487
Sbjct	470	GAGGTAGTGACAAGAAATAACAATCCGTGTCCATATTCTGGATGCGAAATTGGAATGAGT	529
Query	488	ACAATTTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG	547
Sbjct	530	ACAATTTAAATCCTTTAACGAGGACCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG	589
Query	548	TAATTCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGAT	607
Sbjct	590	TAATTCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGAT	649

Query	608	TTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTTCTTCG	667
Sbjct	650	TTCGGGATGGGCCAGTCGGTCTGCCGCAAGGTATGTTACTGGCTGGTCTGTCTTCTTCG	709
Query	668	CAAAGACTGCGTGTGCTCTTAATTGAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAA	727
Sbjct	710	CAAAGACTGCGTGTGCTCTTAAGTGTGCGTAGGATTTGCGACGTTTACTTTGAAAA	769
Query	728	AATTAGAGTGTTCAAAGCAGGCTGTCCGCTTGAATACATGAGCATGGAATAATGGAATAG	787
Sbjct	770	AATTAGAGTGTTCAAAGCAGGCTAACCGCTTGAATACATGAGCATGGAATAATGGAATAG	829
Query	788	GACTTTGGTCTATTTTGTGGTTTCTGGAACCGAAGTAATGATTAAGAGGGACAGTTGG	847
Sbjct	830	GACTTTGGTCTATTTTGTGGTTTCTAGAACTGAAGTAATGATTAAGAGGGACAGTTGG	889
Query	848	GGGCATTTCGTATTTTCGTTGTGAGAGGTGAAATTCCTGGATTACGAAAGACGAACAACCTG	907
Sbjct	890	GGGCATTTCGTATTTTCGTTGTGAGAGGTGAAATTCCTGGATTACGAAAGACGAACAACCTG	949
Query	908	CGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGAC	967
Sbjct	950	CGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGAC	1009
Query	968	GATCAGATACCGTCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTA	1027
Sbjct	1010	GATCAGATACCGTCTAGTTCTAACCATAAACGATGCCGACTAGGGATCAGCGGGCGTTA	1069
Query	1028	TTTTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTAT	1087
Sbjct	1070	TTTTATGACCCCGTTGGCACCTTATGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTAT	1129
Query	1088	GGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGC	1147
Sbjct	1130	GGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGC	1189
Query	1148	GGCTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAG	1207
Sbjct	1190	GGCTTAATTTGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGATTGACAG	1249
Query	1208	ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAG	1267
Sbjct	1250	ATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAG	1309
Query	1268	TGATTGTCTGGTTAATTCGGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGA	1327
Sbjct	1310	TGATTGTCTGGTTAATTCGGTTAACGAACGAGACCTTATCCTGCTAAATAGTCACACGA	1369
Query	1328	ATCTCGCTTCGTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGC	1387
Sbjct	1370	ATCTCGCTTCGTGCCTGACTTCTTAGAGGGACTGTTGGTGTAAACCATCGTAAGGAAGGC	1429
Query	1388	AATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGATG	1447
Sbjct	1430	AATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGATG	1489
Query	1448	GCAGCGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATG	1507
Sbjct	1490	TCAACGAGTCTTAACCTTCGCCGATAGGTGTGGGTAATCTTGTCAAACATCGTCGTGATG	1549
Query	1508	GGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATC	1567
Sbjct	1550	GGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAGCGCGAGTCATC	1609
Query	1568	AGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGA	1627
Sbjct	1610	AGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGA	1669
Query	1628	ATGGTCTAGCGAGACCTTCGGATTGGCACCCTCCGGCCGCAACGGCTGAGGTGGAACG	1687
Sbjct	1670	ATGGTCTAGCGAGACCTTCGGATTGGCACCCTCCGGCCGCAACGGCTGAGGTGGAACG	1729
Query	1688	CCGAGAAGTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGT	1747
Sbjct	1730	CCGAGAAGTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGT	1789
Query	1748	AGGTGAACCTGCGGAAGGATCATTACCGAAA-ACGAGGCTAGCTCTTCCGAGAGC-ATCG	1805
Sbjct	1790	AGGTGAACCTGCGGAAGGATCATTACCGAACTTGCAGTTTATGCTGCAAAAAGCTATTC	1849


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Query 3140 C-ATGCACCTTTGGTTGTTGGCGGCCATATGGCTTCATCCCTACCCCTCGGAACACGGAC 3198
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 3296 CGATGCACCATGGTTGTTGGCGGCCATATGGCTTTATCCGACCCGCTTGAAACACGGAC 3355

Query 3199 CAAGGAGTATATCATGTGCGCAAGTCTTGCGGTGATCGAATCCCAAAGGCACATGAAAG 3258
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 3356 CAAGGAGTCTAACATGTGCGCAAGTCTTGGGGTGACCGAAACCCAGAGGCGCAATGAAAG 3415

Query 3259 TAAAGGCTGCCT--GGCAGCCGAGGTTAGATCTTCGTTCTGCGCGTTTGAAGCGCATCAT 3316
          ||||||| ||| | ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 3416 TGAAGGCTTCCTTGTGAGGCTGAGGTGAGATCTTTGCTCTACGCGAGTGAGGCGCATCAT 3475

Query 3317 CGACCGACCTATTCTACTCTTAGAAAGGTTTGAGTAAGAGCGCATCTGTTGGGACCCGAA 3376
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 3476 CGACCGACCTATTCTACTCTTAGGAAGGTTTGAGTAAGAGCGTACATGTTGGGACCCGAA 3535

Query 3377 AGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACT-AAAAGGAAGCTCGTAG 3435
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct 3536 AGATGGTGAACATATGCCTGAGTAGGGTGAAGCCAGAGGAAACTCTGGTGAAGCTCGTAG 3595

Query 3436 CGATTCTGACGAGCAAATCGATCGTCGAAC 3465
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Sbjct 3596 CGATTCTGACGTGCAAATCGATCGTCGAAC 3625

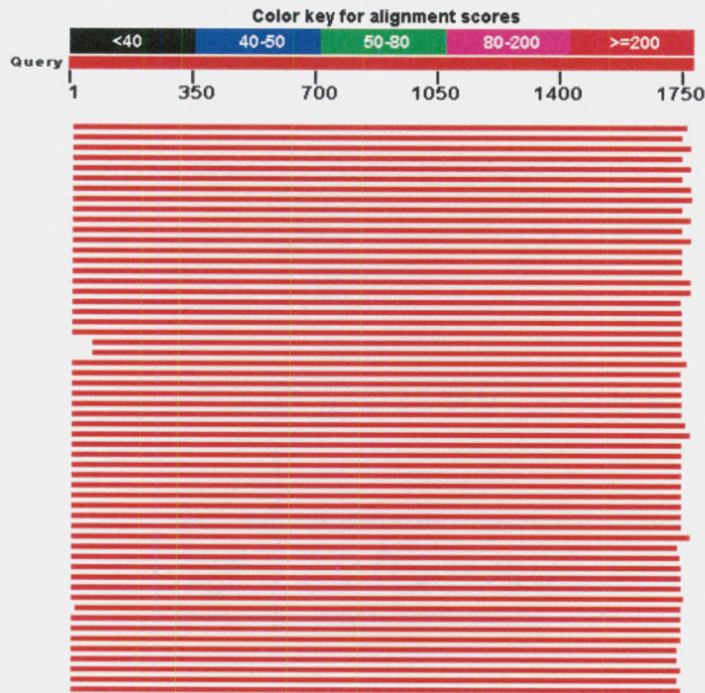
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Figure 37: BLASTn match of Contig1, shown in A. The highest match is with *Aurelia* sp., a maximum identity of 92%, shown in B. Range 1 shows 92% identity. Range 2 shows 83% identity. Note there is a gap in the first result, *Aurelia*.

Individual BLASTn searches were run for each segment of the 45S rDNA cassette to indicate the amount of homology of each region among species. It was not possible to analyze the entire Contig1 as there is only one other cnidarian completely sequenced (*Aurelia*). Therefore, individual segments were analyzed further.

The 18S fragment from Contig1a resulted in a score of ≥ 200 with a maximum identity of 99% with *Chrysaora* sp., demonstrating high levels of homology among other jellyfish (Fig. 38). 18S Contig1a nts align well with 5' and 3' ends of *Chrysaora quinquecirrha* (HM01526), with a 1305 bp gap in between the two aligned regions (Fig. 39). Using Clustal Omega, a phylogenetic tree was predicted by aligning 18S sequence data among different cnidarians. *Chrysaora quinquecirrha* of Barnegat Bay is predicted to be most closely related to other scyphozoan species, as expected (Fig. 40).

A.

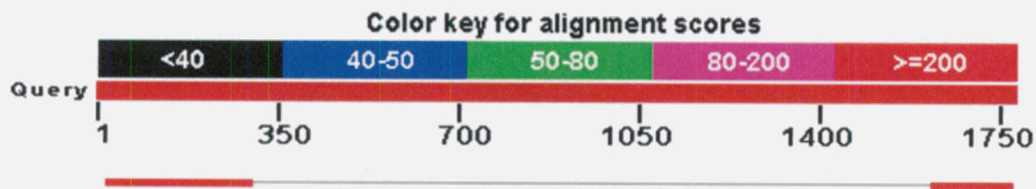


B.

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/>	Chrysaora sp. AOC-2005 small subunit 18S ribosomal RNA gene, partial sequence	3048	3048	98%	0.0	99%	AY920769.1
<input type="checkbox"/>	Chrysaora lactea isolate MOD14810X 18S small subunit ribosomal RNA gene, partial sequence	3000	3000	97%	0.0	98%	HM194810.1
<input type="checkbox"/>	Chrysaora colorata small subunit ribosomal RNA gene, partial sequence	2973	2973	98%	0.0	98%	AF358098.1
<input type="checkbox"/>	Chrysaora melanaster isolate MOD14811Y 18S small subunit ribosomal RNA gene, partial sequence	2960	2960	97%	0.0	98%	HM194811.1
<input type="checkbox"/>	Chrysaora melanaster small subunit ribosomal RNA gene, partial sequence	2957	2957	98%	0.0	97%	AF358099.1
<input type="checkbox"/>	Chrysaora fuscescens isolate MOD14619Q 18S small subunit ribosomal RNA gene, partial sequence	2929	2929	97%	0.0	98%	HM194815.1
<input type="checkbox"/>	Cyanea sp. AOC-2001 small subunit ribosomal RNA gene, partial sequence	2919	2919	98%	0.0	97%	AF358097.1
<input type="checkbox"/>	Aurelia sp. Incheon-2006 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, 28S	2911	2911	99%	0.0	97%	EU278014.1
<input type="checkbox"/>	Sanderia malayensis isolate MOD13625A 18S small subunit ribosomal RNA gene, partial sequence	2897	2897	97%	0.0	97%	HM194809.1
<input type="checkbox"/>	Stromolochus meleagris small subunit ribosomal RNA gene, partial sequence	2888	2888	98%	0.0	96%	AF358101.1

Figure 38: BLASTn match of 18S nucleotides from Contig 1a, position 1-1772, shown in A. B lists the top 10 matches with % maximum identity.

A.



B.

Range 1:

Query 24 GTCTAAGTAGAAGCACTAGCACATTG-AACTGCGAATGGCTC---GGA-GAG-AACCGGT 77

Sbjct 57 GTCTAAGTATAAGCACTTGTACTGTGAACTGCGAATGGCTCATTAAATCAGTTATC-GT 115

Query 78 TTATTTGATTGTACCTTACTACATGGATAACCGWGGTAATTCTAGAGCTAATACATGCGA 137

Sbjct 116 TTATTTGATTGTACCTTACTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCGA 175

Query 138 AAAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTGCTTCG 197

Sbjct 176 AAAGTCCCGACTTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACGGGTGCTTCG 235

Query 198 GTGCCCCGTTTCATGTGGTGATTTCATGATAAATTCTCGAATCGCATGGCCTTGTGCCGCGCA 257

Sbjct 236 GTGCCCCGTTTCATGTGGTGATTTCATGATAAATTCTCGAATCGCATGGCCTTGTGCCGCGCA 295

Query 258 TGTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTG 305

Sbjct 296 TGTTCATTCAAATTTCTGCCCTATCAACTGTCGATGGTAAGGTAGTG 343

Range 2:

Query 1610 GTCGCTACTACCGATTGAATGGTCTAGCGAGACCTTCGGATTGGCACCCTCCGGCCGCGC 1669

Sbjct 361 GTCGCTACTACCGATTGAATGGTCTAGCGAGACCTTCGGATTGGCACTGCTCCGGCCGCGC 420

Query 1670 AACGGCTGAGGTGGAACGCCGAGAAGTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAAG 1729

Sbjct 421 AACGGCTGAGGTGGAACGCCGAGAAGTCGTTCAAGTTCGATCATTTAGAGGAAGTAAAAG 480

Query 1730 TCGTAACAAGGTTT-CCGTAGGTGAACCTGCGGAAGGATC 1768

Sbjct 481 TCGTAACAAGGTTTACCGTAGGTGAACCTGCGGAAGGATC 520

Figure 39: BLASTn match of 18S nucleotides of Contig1a to *Chrysaora quinquecirrha* (HM015266), 18S ribosomal RNA gene, partial sequence (Range 1: 94%, Range 2: 98% identity).

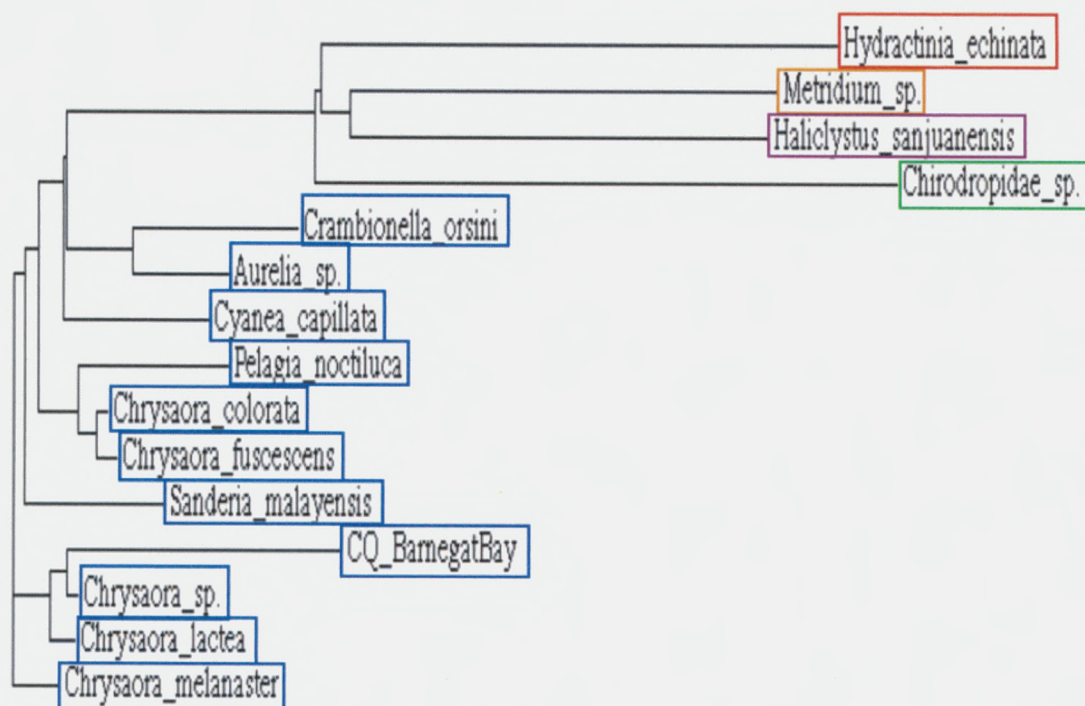
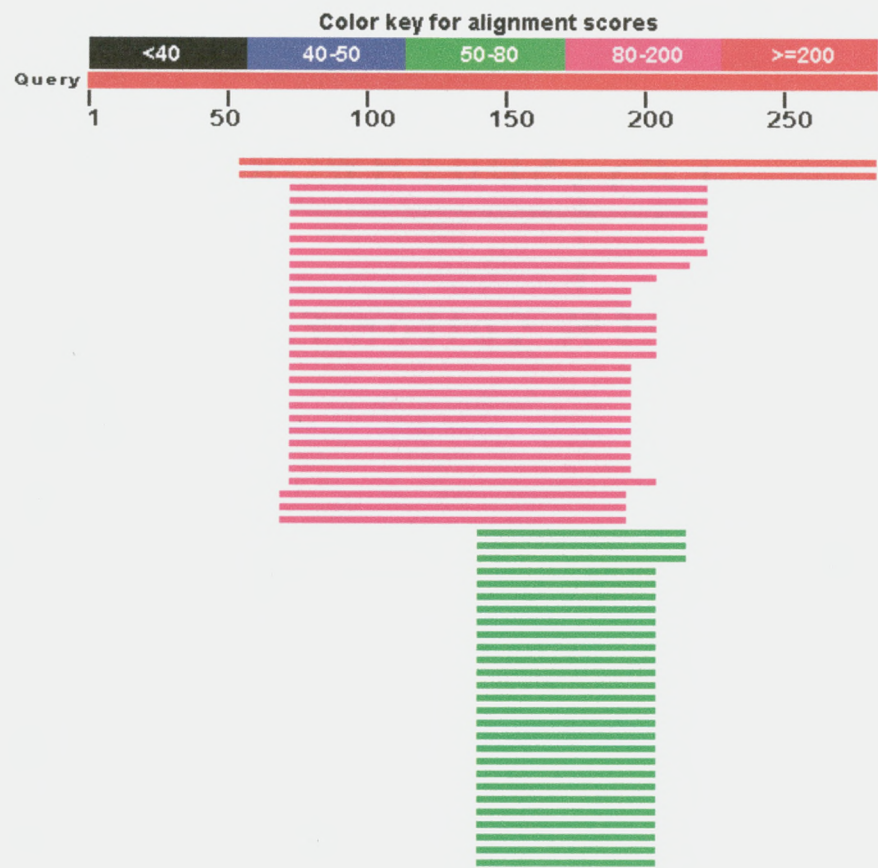


Figure 40: Phylogenetic Tree from Clustal Omega using 18S cnidarian sequences. Comparison of 18S sequences among cnidarians. This figure demonstrates *C. quinquecirrha* of Barnegat Bay to be most closely related to *Chrysaora sp.* Blue denotes Class Scyphozoa; Red denotes class Hydrozoa; Orange denotes class Anthozoa; Purple denotes class Staurozoa; Green denotes class Cubozoa.

When ITS1 nts from Contig1a were analyzed through a BLASTn search, the maximum scores ranged from 50 - ≥ 200 (Fig. 41). The highest match is with *Crambionella stuhlmanni*, a scyphozoan jellyfish species that belongs to the family Rhizostomeae. Of the 284 bp identified in Contig1a, only 201 nts matched with *C. stuhlmanni*. Other matches include *Aurelia*, *Cyanea*, and *Pelagia* species. Also, one *Chrysaora* sample exhibited a 77% maximum identity, of which only 95 nts matched with one another. Lower % identities is to be expected as each ITS1 sequence shows high levels of variation between species. A clustal alignment indicates conserved regions and regions with high variability between species (Fig. 42a). The phylogenetic tree

predicts that *C. quinquecirrha* of Barnegat Bay and Navesink River are most closely related to one another (Fig. 42b).

A.



B.

Description	Max score	Total score	Query cover	E value	Max ident	Accession
Crambionella stuhlmanni voucher UWC lot CS0014 18S ribosomal RNA gene and internal transcribed spacer 1, partial sequence	269	269	80%	7e-69	87%	HM348774.1
Crambionella stuhlmanni voucher UWC lot CS0013 18S ribosomal RNA gene and internal transcribed spacer 1, partial sequence	269	269	80%	7e-69	87%	HM348773.1
Uncultured eukaryote clone N201T_245 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	111	111	52%	4e-21	78%	GU941284.1
Uncultured eukaryote clone N201T_238 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	111	111	52%	4e-21	78%	GU941281.1
Uncultured eukaryote clone N201T_236 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	111	111	52%	4e-21	78%	GU941280.1
Uncultured eukaryote clone N201T_205 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	111	111	52%	4e-21	78%	GU941271.1
Uncultured eukaryote clone N201T_240 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	109	109	52%	1e-20	78%	GU941282.1
Uncultured eukaryote clone N201T_232 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	107	107	52%	5e-20	77%	GU941278.1
Aurelia sp. MCA 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, complete sequence, and 5.8S ribosomal RNA gene	100	100	50%	7e-18	75%	AF461412.1
Cyanea capillata 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 5.8S ribosomal RNA gene	100	100	46%	7e-18	79%	U65481.1

Figure 41: BLASTn match of ITS1 from Contig1a, position 1773-2056, shown in A. B lists the top 10 matches with % maximum identity.

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

-----ATTACCGTTGGAGTA-----
 -----GTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAATACGCGTACCTCGCC
 TAACAAGGTTTCGTAGGTGAACCTGCGGAAGGATCATTACCGAATAGGTTGGAAGGAG
 -----GGAAGGATCATTACCGATCGTGTTCCTA-----
 -----GGTTTCGTAGGTGAACCTGCGGAAGGATCATTACCGAAGTTGG-----
 -----CGAAACGAGGCTAGCTCT-----
 -----TTATTTTATAGGTGAACCTGCGGAAGGATCATTACCGA AACGAGCTAGCTCT
 ★★

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

-----CGGTTGATCGTG-----CAATTATGATGCGCTAGCACACCACTGATACACTG
 GAGTCGCAAGGCCAAGCAGA-----CGTACCATGATACATGCCATTGATCTACATCTCACTG
 TCGGCTCCCTCTCGAAGTTTAGTGTGATCGGTGTAAACAACGCCAGCAACATGACTACTG
 -----A-----CACACAGCTGCTACTGCTTCCGCAAGTTG-----
 -----CTACACGCTAT-----CAACCCCTTGACTGACGCTCAAGGATATGCGAACCACTG
 TCCGAGAGCATCGAATGACT-----CTTGATCGACCGGGTCGTCAAGGCTATGCGAACCACTG
 TCCGAGAGCATCGAATGACT-----CTTGATCGACCGGGTCGTCAAGGCTATGCGAACCACTG

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

[illegible]

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

[illegible]

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

TCATTATGTCCTTTGAATACTTTATGAATATTTCTAAATAAAAGGTGAACACATCTGTT
TGCGTTTCCAAATATACTTTCAACAATCTTCTCTCGACATTCGCGACCGCATCTTTGCTG
AACAGAGTTTGTCTTTTCAACAAACAAAAAACCAATTCG---TCACAATTGTATTCCTG
TAGTTGTTTTTCAAAACTCTTTTAAAACCTCCAGAATT---CTAGCTCTCAACACTGTT
TAGTGTTATATTTTTCAAACTGAAATCATGAATTCA---TAATTATTGACACATGAT
GAGTGTTTTATTTTTCAAACTGAAATTCATGAGTTGG---AATTGTTGGCTTTTTTCA
GAGTGTTTTATTTTTCAAACTGAAATTCATGAGTTGG---AATTGTTGGCTTTTTTCA

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

GTGTG--TGAGCCATGTGAGAGAAAATAGA--
AGCTGGTCAGATAAATCATAAACATGAG--
ATCTAGTCGAGCAGCTGTTCAAGCTGAGCAAGCTGTTCTGATTCTGTTGAGATGAAAAAT
GGGTCAGTTAGAAGAAAGGAAGAAGT
--TGTGAATAGTTGGAATGAAAAAGTCAGA--
AAGTCAATAGTTGGAATGAAAAGTCAGAGA--
AAGTCAATAGTTGGAATGAAAAGTCAGAGA--

Chrysaora_sp.
Cyanea_capillata
Aurelia_sp.
Pelagia_noctiluca
Crambionella_stuhlmanni
CQ_BarnegatBay
CQ_NavesinkRiver

GAGAT

B.

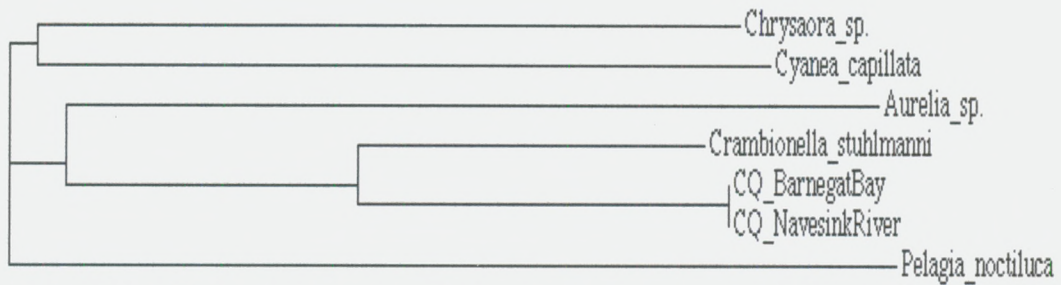


Figure 42: ITS1 Clustal alignment of 7 scyphozoan species with Contig1a, shown in A. Predicted phylogenetic tree based on ITS1 data, shown in B.

When 5.8S from Contig1a was analyzed, the maximum score was ≥ 200 with a maximum identity of 98% (Fig. 43). There is very little sequence data for 5.8S among scyphozoans, and zero data for *Chrysaora* species. The highest match of our 5.8S sequence was with *Aurelia*. Other matches include *Cyanea*, *Palythoa*, *Sphenopus*, and *Zoanthus*, demonstrating high levels of homology among species.

A.



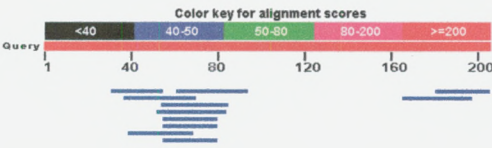
B.

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/>	Aurelia sp. Geio-do-2006 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2	269	269	99%	3e-69	98%	EU332745.1
<input type="checkbox"/>	Aurelia sp. Busan-2007 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2	269	269	99%	3e-69	98%	EU332744.1
<input type="checkbox"/>	Aurelia sp. Incheon-2008 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, 28S ribosomal RNA gene, and internal transcribed spacer 1	269	269	99%	3e-69	98%	EU276014.1
<input type="checkbox"/>	Aurelia sp. 9-MND-2005 clone MDJ12_AM2a internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935216.1
<input type="checkbox"/>	Aurelia sp. 1-MND-2003 clone #1 MiyazuBay internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935214.1
<input type="checkbox"/>	Aurelia sp. 7-MND-2005 clone BapofSton1 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935213.1
<input type="checkbox"/>	Aurelia sp. 7-MND-2005 clone North Adriatic2 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935212.1
<input type="checkbox"/>	Aurelia sp. 5-MND-2003 clone Croatia_Mille4 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935210.1
<input type="checkbox"/>	Aurelia aurita clone Blomsund20 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935206.1
<input type="checkbox"/>	Aurelia sp. 1-MND-2003 clone California_MdR4 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	269	269	99%	3e-69	98%	AY935203.1

Figure 43: BLASTn match of 5.8S, from Contig1a, position 2057-2214, shown in A. Top 10 matches are shown in B. The highest match is with *Aurelia*.

When ITS2 from Contig1a was analyzed through a BLASTn search, the maximum scores were low and ranged from 40-60 (Fig. 44). Not enough data was available on the GenBank database to predict phylogenetic relationships using the ITS2 region at this time.

A.



B.

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/>	Chilo suppressalis Unigene18045: All transcribed RNA sequence	44.6	44.6	11%	0.34	100%	GAJ501018037.1
<input type="checkbox"/>	Gordoniabacter pamelaeeae 7-10-1-b draft genome	42.8	42.8	16%	1.2	88%	FP929047.1
<input type="checkbox"/>	Uncultured soil bacterium clone B128 VEG gene cluster, complete sequence	42.8	42.8	15%	1.2	90%	EU874252.1
<input type="checkbox"/>	Pyrodictum bahamense var. compressum PbaiaRW0_c5304 transcribed RNA sequence	41.0	41.0	12%	4.1	96%	GAIQ01038327.1
<input type="checkbox"/>	Alexandrium tamarense Atam94712 transcribed RNA sequence	41.0	41.0	16%	4.1	88%	GAB01008412.1
<input type="checkbox"/>	PREDICTED: Taeniopogon guffata UTP18 small subunit (SSU) processome component homolog (yeast [UTP18], mRNA)	41.0	41.0	15%	4.1	88%	XM_002191749.2
<input type="checkbox"/>	Amycolatopsis orientalis vancomycin biosynthesis cluster, strain ATCC19795	41.0	41.0	12%	4.1	96%	HE588771.1
<input type="checkbox"/>	Uncultured organism CA915 oligopeptide biosynthetic gene cluster, complete sequence	41.0	41.0	12%	4.1	96%	HM486076.1
<input type="checkbox"/>	Pseudomonas stutzeri A1501, complete genome	41.0	41.0	14%	4.1	90%	CF000304.1
<input type="checkbox"/>	Nitrosococcus oceanus ATCC 19707, complete genome	41.0	41.0	15%	4.1	88%	CF000127.1
<input type="checkbox"/>	Amycolatopsis orientalis glycosyltransferase GME (gme) gene, complete cds	41.0	41.0	12%	4.1	96%	AF351622.1

Figure 44: A BLASTn match of ITS2, from Contig1a, position 2215-2419 shown in A. Top 10 matches shown in B.

28S sequence data was analyzed previously as consensus sequence 4 (Fig. 22-23). When a phylogenetic tree was predicted by aligning 28S sequences from other cnidarians

using Clustal Omega, results were almost identical to the 18S phylogenetic tree seen in Fig. 40. *C. quinquecirrha* of Barnegat Bay is most closely related to other scyphozoan species, the highest relationship with *Chrysaora* sp. (Fig. 45).

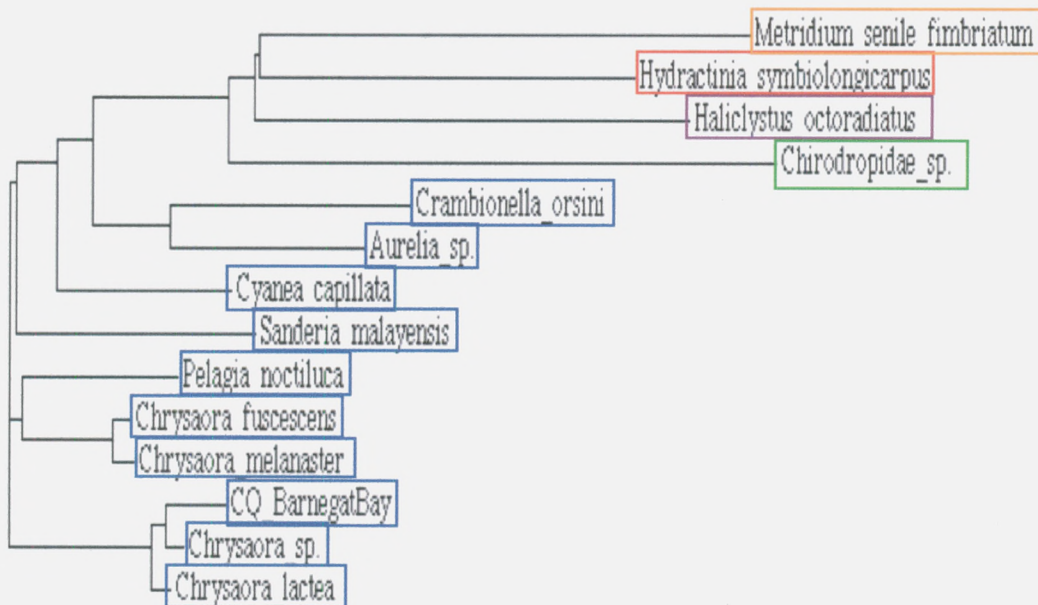


Figure 45: Phylogenetic Tree from Clustal Omega using 28S cnidarian sequences. Comparison of 28S sequences among cnidarians. Blue denotes Class Scyphozoa; Red denotes class Hydrozoa; Orange denotes class Anthozoa; Purple denotes class Staurozoa; Green denotes class Cubozoa.

Primer Set	Forward	Reverse
1	Primer 1 - 18SF CTCGTAGTTGGATTTCGGGA	Primer 2 - 18SR AACTAAGAACGGCCATGCAC
2	Primer 3 - 18SF AACCTGGTTGATCCTGCCAGT	Primer 2 - 18SR AACTAAGAACGGCCATGCAC
3	Primer 1 - 18SF CTCGTAGTTGGATTTCGGGA	Primer 4 - 18SR GATCCTTCTGCAGGTTACCTAC
4	Primer 5 - 28SF ACCCGCTGAATTTAAGCATA	Primer 8 - 28SR GAAACTTCGGAGGGAACCAGCTAC
5	Primer 1 - 18SF CTCGTAGTTGGATTTCGGGA	Primer 12 - ITS1R CGCACGAGCCGAGTCCACCTTAGAAG
6	Primer 13 - 18SF TAGGTGAACCTGCGGAAGGA	Primer 14 - 28SR TATGCTTAAATTCAGCGGGTAG

Table 5: Summary of Primer Sets used in this study.

Consensus Sequence	
1	Barnegat Bay – Set 1 (internal 18S fragment)
2	Chesapeake Bay – Set 1 (internal 18S fragment)
3	Barnegat Bay – Sets 1-3 (18S)
4	Barnegat Bay - Set 4 (28S)
5	Barnegat Bay - consensus 3 and Set 5 (18S, ITS1)
6	Barnegat Bay – Set 6 (ITS1, 5.8S, ITS2)
7	Navesink River – Set 6 (ITS1, 5.8S, ITS2)

Table 6: Summary of consensus sequences.

Contig	
1a	Barnegat Bay (18S, ITS1, 5.8S, ITS2)
1b	Barnegat Bay (28S)

Table 7: Summary of Final Contig sequences.

Discussion

Attempting to identify a sequence almost 8,000 bp long with little to no reference in cnidarian databases requires conserved regions. Since the 18S, 5.8S, and 28S rDNA sequences exhibit highly conserved regions, these regions were identified through ClustalW alignments to establish primer sets. Previously identified ITS1, ITS2, and IGS regions were not analyzed to design primer sets for two reasons: these regions are highly variable and show little areas of homology, and secondly, there is very little information on cnidarian sequences. In addition, due to the length of the cassette, it was necessary to design multiple primer sets that would PCR amplify short fragments that could then be assembled together into a final consensus sequence. Primer sets 1-6 were used to establish Contig1, where primer sets 1-3 identified 18S fragments, primer set 4 identified 28S fragments, primer set 5 identified 18S and ITS1 fragments, and finally primer set 6 identified the gap between 18S and 28S all in *C. quinquecirrha* of Barnegat Bay.

This study determined approximately 44.8% of the 45S rDNA cassette of *C. quinquecirrha*. The complete 45S rDNA cassette of *Aurelia* was used as a reference to indicate the percentage of the *C. quinquecirrha* cassette identified in Contig1. In *Aurelia*, the cassette was determined to be 7731 bp long. Contig 1 is 3465 bp long. This contig contains 18S rDNA, ITS1, 5.8S rDNA, ITS2, and 28S rDNA sequences.

Position 1-1772 in Contig1a (1772 nts total) matched with position 42-1814 in *Aurelia*, indicating this region as 18S rDNA. In comparison to *Aurelia*, 96% of the 18S rDNA was determined. The missing 4% of the sequence includes the first 42 nts at the 5' end of the 18S rDNA sequence. Even though the complete sequence was not identified, enough sequence data was generated to draw comparisons among species. The 18S

rDNA provides supporting evidence that *C. quinquecirrha* is most closely related to other *Chrysaora* species. Also, in comparison to cnidarian classes, *C. quinquecirrha* is most closely related to species within its class Scyphozoa, as expected.

At position 1773-2056 (284 nts total) in Contig1a, there was alignment with the ITS1 region in *Aurelia* located at position 1815-2086 (272 nts total). 100% of the ITS1 region was identified in this study and it appears that this region is 14 nucleotides longer with a higher % GC content in *C. quinquecirrha* (284 nts, 47.9% GC content) as compared to *Aurelia* (272 nts, 39.7% GC content). This is recognized because nts in Contig1a matched with the 3' end of the 18S rDNA and the 5' end of the 5.8S rDNA, which are conserved regions. The number of nts between these two conserved regions was 284, the ITS1 region. In comparison to other cnidarians, the length of ITS1 and % GC content was slightly higher than the average of 253.9 bp with 45.6% GC content (Chow et al. 2009). In a phylogenetic analysis of the ITS1 region among different scyphozoan species, *C. quinquecirrha* of Barnegat Bay was most closely related to *C. quinquecirrha* of Navesink River. This is to be expected as even though they are two populations of sea nettles, they belong to the same species. The next closest relative was *Crambionella stuhlmani*. The validity of this estimation is not clear as there was very little scyphozoan ITS1 data to draw comparisons. In fact, only one other *Chrysaora* sample was used in comparison. More ITS1 data needs to be compiled for *Chrysaora* species.

Position 2057-2214 (158 nts total) in Contig1a matched with position 2087-2244 in *Aurelia*, indicating this region as 5.8S rDNA. 100% of the 5.8S rDNA was determined in this study. It is clear that the 5.8S rDNA sequence in both species is the same length

with high levels of homology. Again, very little data is currently known on any database for 5.8S rDNA in cnidarians. At this point, there is little value in using 5.8S rDNA for phylogenetic studies until more 5.8S rDNA in cnidarian species are identified.

In Contig1a, position 2215-2419 (205 nts total) is the ITS2 region, as it matched with position 2245-2522 in *Aurelia*. 100% of the ITS2 region was determined in this study. The ITS2 region showed very little homology with any other species, however, almost no cnidarian ITS2 information is available on any database currently. In comparison to *Aurelia* (278 bp, 51.4% GC content), *C. quinquecirrha*'s ITS2 region is much shorter (73 nts less) with a higher 56.1% GC content. Being that the ITS regions are highly variable, the ITS regions may be able to be used as a marker to identify a species. It would be interesting to compare the ITS regions with other *Chrysaora* species in future experiments.

In Contig1b, position 1-1046 (1046 nts total) matched with position 2587-3633 in *Aurelia*, indicating this region as 28S rDNA. By comparing Contig 1a and 1b to *Aurelia*, there is a gap between Contig 1a and 1b of approximately 65 bp at the 5' end of 28S. Therefore, 29% of the 28S rDNA sequence was determined in this study. Even though the complete sequence was not identified, enough sequence data was generated to draw comparisons among species. Similar to 18S rDNA analyses, 28S rDNA provides supporting evidence that *C. quinquecirrha* is most closely related to other *Chrysaora* species. Also, in comparison to cnidarian classes, *C. quinquecirrha* is most closely related to species within its class Scyphozoa, as expected.

At this point, 18S and 28S rDNA sequences are most useful in predicting phylogenetic relationships. In the future as more reference sequences are available, the ITS regions may become of value in predicting phylogenetic relationships. But, at this point, the ITS regions do not provide any correlations in predicting phylogenetic relationships.

This study focused on populations of *C. quinquecirrha* in Barnegat Bay (Contig 1). However, DNA samples from Chesapeake Bay and Navesink River were also analyzed. Only one out of the two DNA samples available from Chesapeake Bay generated bands for primer set 1. No results were observed in primer sets 2-6. In a comparison between consensus 1 (*CQ* Barnegat Bay) and 2 (*CQ* Chesapeake Bay) which consisted of an internal fragment of 18S rDNA, there was 99% maximum identity. Out of the 640 bp fragment, only 2 nts differed. It is unclear as to why primer sets 2-6 did not work with this DNA sample. It is possible that *C. quinquecirrha* of Chesapeake Bay does not contain the conserved regions found in *C. quinquecirrha* of Barnegat Bay and other cnidarians. *Chrysaora quinquecirrha* of Chesapeake Bay may be very similar to Barnegat Bay populations, however, this will require more sequence analysis. The design of new primer sets for successful amplification need to be developed. Successfully amplifying the ITS regions in *C. quinquecirrha* of Chesapeake Bay would provide the best comparison as these are the most highly variable regions.

The only primer set that worked for Navesink River DNA samples was primer set 6, which amplified the ITS regions and 5.8S rDNA. In a comparison between consensus sequence 6 (*CQ* Barnegat Bay) and 7 (*CQ* Navesink River), there was 99% maximum identity. Only one nucleotide differed between sequences. This is very interesting

because ITS regions are variable among species. Since these two sequences are nearly identical, this provides supporting evidence of how related the two populations are to one another. It is currently unclear as to how *C. quinquecirrha* populations developed in the Navesink River, but this data suggests that they originated from Barnegat Bay populations.

In comparison of Contig1 to the partial 18S rDNA sequence of *C. quinquecirrha* (HM01526) published on GenBank database, the partial sequence aligns well with nucleotides at the 5' end and 3' end of 18S from Contig1a. Our sequence is 1772 bp long, whereas the previously identified sequence is only 520 bp long. There is a 1305 bp gap in between the two aligned regions (Fig. 39), demonstrating that the partial sequence has a major deletion of the 18S rDNA. We are confident that our sequence is correctly identified as we used multiple individual *C. quinquecirrha* samples from Barnegat Bay all with the same results. There are two likely possibilities as to how the partial sequence was identified. The individual sample collected could have a unique DNA sequence unlike all other *Chrysaora* organisms and contain a major deletion of the 18S rDNA sequence. However, it is more likely that when this 520 bp sequence was created, the 5' end and 3' end were sequenced and connected together manually. The extension time used in amplification was most likely too short, therefore not allowing for amplification of the entire fragment. Both the 5' end and 3' end would be amplified under these conditions, but amplification would be cut short, resulting in a shorter sequence.

In conclusion, 44.8% of the 45S rDNA cassette of *Chrysaora quinquecirrha* of Barnegat Bay has been successfully identified in this study. Specifically, 96% of the 18S rDNA, 100% ITS1, 100% 5.8S rDNA, and 29% of the 28S rDNA regions have been

sequenced. The entire IGS region, the first 41 nts at the 5' end of the 18S rDNA, and the remaining 2560 bp of the 28S rDNA have yet to be determined. Further studies with new primer sets need to be identified for further amplification and sequence analysis to complete the cassette. As more ribosomal DNA in cnidarian species are identified, comparisons of 45S rDNA cassettes will add another element when determining evolutionary and phylogenetic relationships. In addition, the ITS regions may serve as useful markers to identify a species and determine the relatedness of populations of jellyfish.

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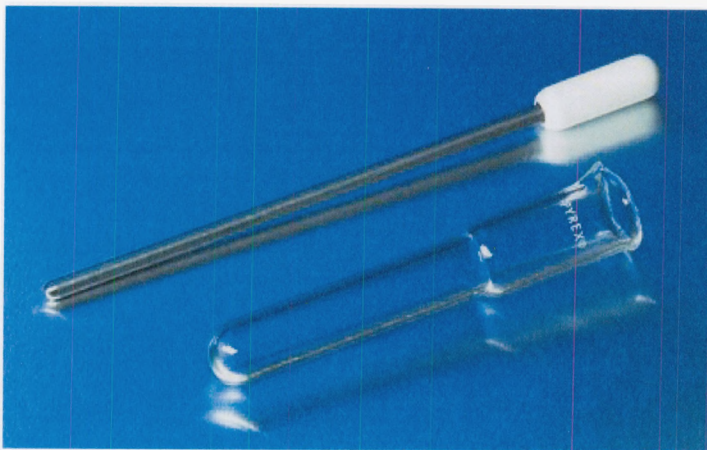
Appendix:

Jellyfish CTAB/NaCl DNA Extraction Protocol

Reagent	[Final]	Volume /weight	# of Samples	Total Volume/ weight	Checklist
CTAB (solid)	2%	10 mg			
ddH ₂ O		289 μ L			
1 M Tris	100 mM	50 μ L			
5 M NaCl	1.4 M	140 μ L			
0.5 M EDTA	20 mM	20 μ L			
β - mercaptoethanol (14.3 M stock)	0.2% (28.6 mM)	1 μ L			
Proteinase K	0.1 mg/mL	50 μ g			
Single = Total 500 μ L					

- Combine CTAB and water in sterile 15 mL plastic tube. Swirl under hot tap water until CTAB has dissolved.
- Add other reagents in order. Move to hood to add the β -mercaptoethanol.
- Add proteinase K powder last. Cap and invert to dissolve.
- Add 500 μ L mix to each sample in 1.5 mL Eppendorf tube.
- Grind each sample separately with blue micropestle, leaving pestle in tube.
- Incubate @ 60°C for 60 minutes. Invert tubes occasionally to mix.
- Add 0.5 mL of chloroform:isoamyl alcohol (24:1).
- Gently mix for 2 minutes by inverting the tube.

- Spin for 10 minutes @ maximum speed (14,000 x g) in microcentrifuge @4°C.
- Transfer upper aqueous phase into new 1.5 mL tube. Do not transfer any solid material at the interface to new tube.
- Add 1 μ L RNase A (10 mg/mL) and incubate 30 minutes @37°C.
- Add 2/3 volume of isopropanol. Close cap and gently invert to mix.
- Allow tube to sit at room temperature for 2 hours to overnight. Watch for formation of DNA fibers in solution.
- Spin for 15 minutes @14,000 x g at 4°C to pellet the DNA.
- Remove supernatant carefully. Then wash 2X with 500 μ L of 70% EtOH. Each time spin for 15 minutes @14,000 x g at 4°C to pellet the DNA.
- Remove supernatant and dry pellet briefly (5 min) in Speed-Vac without heating.
- Resuspend pellet in minimum volume of TE (pH 8.0).
- Determine concentration and purity by UV absorption with NanoDrop.
- Store in aliquots at -20°C.
- Run small aliquot on 1.0% agarose gel to check for quality and size of DNA.



Potter-Elvehjem homogenizer

DNA sequencing analysis of PCR product

1/16X reactions

Single reaction (1/16)		Master mix (10 rxn)
0.5 μ l	Big dye terminator ver. 3.1 RR mix	5.0 μ l
3.75 μ l	5X sequencing buffer	37.5 μ l
1.0 μ l	Sequencing primer (10 μ M)	----
1.0 μ l	Template DNA (PCR product)	----
13.75 μ l	Sterile deionized water	137.5 μ l
20 μ l	Total reaction volume	180 μ l

- Dispense 18 μ l of master mix to each of ten 200 μ l PCR tubes (bubble cap).
- Set up separate reactions for forward and reverse primer.
- Mix, spin and amplify on Veriti Thermocycler. Run Big Dye Kit Standard Program.

Clean up of sequencing reactions:

- Add 16 μ l of sterile deionized water. Add 64 μ l of 100% EtOH at room temperature (RT). Mix by inversion. Transfer to 1.5 ml Eppendorff tubes. Let stand at RT for 15 min.
- Spin at RT for 20 min. at maximum G. Dump supernatant- blot upside down on kimwipe/paper towel.
- Add 250 μ l of 70% EtOH at RT. Mix by inversion. Spin for 10 min at max. G.
- Dump supernatant. Dry for 5 min in speed vac.
- Add 20 μ l of Hi-Di formamide. Vortex, spin briefly.
- Heat at 95 C for 2 min. Keep in ice for 1 min.